

For Reference

NOT TO BE TAKEN FROM THIS ROOM

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex LIBRIS
UNIVERSITATIS
ALBERTAENSIS



THE UNIVERSITY OF ALBERTA

VERNALIZATION STUDIES ON CEREALS

by

EDWARD STEPHEN REDSHAW



A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA

MARCH, 1968

UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Vernalization Studies on Cereals" submitted by Edward Stephen Redshaw in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The objectives of this investigation were threefold: to determine if the application of extracts from vernalized tissue to unvernallized would induce earlier flowering, to determine if application of protein and nucleic acid antimetabolites to vernalizing seedlings would delay flowering and to compare the changes in lipid composition of seedlings of spring and winter varieties during vernalization treatment.

No effect on number of days to heading of unvernallized Sangaste fall rye was observed following treatment with vernalized tissue extracts and nucleic acids. Applications of protein and nucleic acid antimetabolites to vernalizing and vernalized Sangaste fall rye did not markedly affect the vernalization response or impede progress to flowering. There were significant differences between various treatments but no overall pattern emerged. There were marked morphological effects which suggested that vegetative growth was affected without any noticeable effect on reproductive growth. Noticeable changes in lipids were observed during growth of Sangaste fall rye, Prolific spring rye, Kharkov winter wheat, and Red Bobs spring wheat, at vernalizing temperature, over a period of 6 weeks. There was, however, little difference between the trends exhibited by the 4 varieties, apart

from the fact that the rye varieties apparently accumulated more linolenic acid than the wheat varieties, whereas the reverse was true for linoleic acid. The results suggested that the lipids under study did not play a direct role in the vernalization response, and the changes observed were a result of low temperature growth.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. Saul Zalik for his guidance throughout the course of my research program, and during the preparation of this manuscript. Also, I wish to thank Mr R Berg of the Department of Genetics for supplying the seed material used in this study. The actinomycin D was kindly supplied by Merck, Sharp and Dohme of Canada Limited, the chloramphenicol by Parke, Davis and Company Limited, and the 5 - fluorouracil and 5 - fluorodeoxyuridine by Hoffman - La Roche Incorporated.

Thanks are also due to Dr. D. Hadžijev with whom discussion always led to a clarification of many problems concerning the extraction and fractionation of lipids. Special thanks are extended to Mr M. Stewart, Mr L. Thomson and Mrs R. Craig for their assistance in carrying out the various extractions and analyses, and to Mr B. Zytaruk for photographing the thin layer chromatography plates.

I am extremely grateful to Miss V. Dirk for her typing of the thesis. To my wife, Betty, I should like to express my gratitude for her patience and understanding during my years at university.

Thanks are expressed to the University of Alberta, the Department of Plant Science, the Province of Alberta, and the National Research Council of Canada for the financial assistance extended to me, and especially for the award of the University of Alberta Dissertation Fellowship in my final year.

TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	1
LITERATURE REVIEW	
1. Vernalization	4
2. Conditions Affecting Vernalization	5
3. The Vernalization Process	8
a. Hormonal Theory	9
b. Biochemical Changes Theory	19
c. Biophysical Changes Theory	22
Section I. Testing of Method for Effecting Vernalization	
Materials and Methods	24
Results and Discussion	26
Section II. Effects of Vernalized Tissue Extracts and Nucleic Acids on Unvernalized Material	
Materials and Methods	30
a. Vernalized Tissue Extracts	30
b. Nucleic Acids	31
1. Ribonucleic Acid	31
2. Deoxyribonucleic Acid	34
Results and Discussion	38
Section III. Effects of Protein and Nucleic Acid Antimetabolites on Vernalizing and Vernalized Seedlings	
Materials and Methods	41
Results and Discussion	45
Section IV. Changes in Lipids During Vernalization	
Materials and Methods	57

TABLE OF CONTENTS (Con't)

	<u>Page</u>
a. Extraction of Lipids	58
b. Fractionation of Lipids	59
c. Phosphorus Determinations	63
d. Gas Liquid Chromatography	63
e. Thin Layer Chromatography	65
Results and Discussion	
a. Materials and Method	66
b. Vernalization	69
c. Total Lipids	71
d. Phosphorus Determinations	74
e. Gas Liquid Chromatography	77
f. Thin Layer Chromatography	82
GENERAL DISCUSSION AND CONCLUSIONS	83
LITERATURE CITED	88

LIST OF TABLES

	<u>Page</u>
1. Number of days from planting to heading of Sangaste fall rye as affected by soaking and /or germination of seeds prior to vernalization, and by duration of vernalization	25
11.a. Number of days from planting to heading of unvernallized Sangaste fall rye as affected by applications of vernalized plant extracts.	36
11.b. Number of days from planting to heading of unvernallized Sangaste fall rye as affected by applications of nucleic acids	37
111. Effect of protein and nucleic acid antimetabolites on number of days from planting to heading of Sangaste fall rye when applied during and after vernalization..	43
1V. Analysis of variance for the data concerning the effect of protein and nucleic acid antimetabolites on number of days from planting to heading of Sangaste fall rye when applied during and after vernalization..	44
V. Effect of protein and nucleic acid antimetabolites on number of tillers of Sangaste fall rye when applied during and after vernalization	49
VI. Changes in total lipids and lipid fractions of cereal varieties during vernalization	70
VII. Changes in polar lipid phosphorus of cereal varieties during vernalization	73
VIII. Changes in the major fatty acids of the polar lipid fractions of cereal varieties during vernalization	75
IX. Changes in the major fatty acids of the non-polar lipid fractions of cereal varieties during vernalization	76

LIST OF FIGURES

	<u>Page</u>
1. Photograph showing vernalized and unvernallized Sangaste fall rye plants	27
2. Diagram showing a parafilm funnel fitted on the coleoptile of a seedling	29
3. Spectrum of RNA isolated from vernalized Sangaste fall rye seedlings by the methods of Laskov <u>et al.</u> (1959) and Littauer and Eisenberg (1959)	33
4. Spectrum of DNA isolated from vernalized Sangaste fall rye seedlings by the method of Lyttleton and Petersen (1964)	35
5. Photograph showing morphological effects produced by applications of chloramphenicol to vernalizing Sangaste fall rye seedlings	46
6. Photograph showing morphological effects produced by applications of 5 - fluorouracil and 5 - fluorodeoxyuridine to vernalizing Sangaste fall rye seedlings	48
7. Photograph showing Sangaste fall rye plants which were treated with actinomycin D while undergoing vernalization	53
8. Spectrum of the phosphomolybdate complex produced during the determination of phosphorus by the method of Bartlett (1958)	61
9. Standard curve for phosphorus determinations. The standards were prepared and determined by the method of Bartlett (1958)	62
10. Relationship of number of days to 100% heading to duration of vernalization of cereal varieties	68
11. Photographs showing examples of the type of results obtained at each stage of vernalization for all cereal varieties by thin layer chromatography of polar and non-polar lipid fractions	81

INTRODUCTION

Vernalization may be defined as the substitution of chilling of germinating seed or plants for the natural low temperature of winter, in order to hasten, or to make possible, the initiation of flower primordia at some later date.

The vernalization technique was early regarded as the solution of the problem of bringing crops to maturity in the short northern summer and of producing crops in drought affected regions. Also, it might provide the means of bringing high cash value crops, such as, market garden or greenhouse plants to maturity in time to catch early markets, or of producing seed in one year from biennial or perennial crops not normally fruiting in their first year.

In an attempt to understand and determine the exact nature of the vernalization response, numerous investigations have been carried out on a variety of plants. Two thorough reviews of the subject have been written recently by Purvis (1961) and Lang (1965). Considerable factual information on vernalization has accumulated, but a clear explanation with convincing evidence for the vernalization mechanism has not emerged.

The aim of this project was to contribute further towards an understanding of the vernalization process.

The first step was to test the method to be used for

effecting vernalization.

The next step was to try and induce earlier heading in unvernallized material by the application of vernalized tissue extracts and nucleic acids. Although numerous investigations have already been carried out in this connection (e.g. Melchers and Lang, 1941; Purvis and Gregory, 1953; Sarkar, 1958; Tomita, 1958, 1959) the results have been variable. It was therefore of interest to determine the response of Sangaste fall rye to applications of these extracts.

This investigation was followed by a study of the effects of protein and nucleic acid antimetabolites on growth and heading when applied to vernalizing and vernalized seedlings. Studies in this area are markedly deficient in the literature, even though there is considerable evidence (Cutter, 1965) that nucleic acids are implicated in the ultimate control of flowering.

The final study was on changes in lipids during vernalization. Apart from reports on changes in total lipids during vernalization of wheat (David, 1947, 1951), rye (Valuta and Brad, 1958), mustard, radish and oats (Duperon, 1949, 1952) no further work has apparently been published in this connection. Most of the present day investigations on cereal lipids are concerned with their role in the baking quality of flour (e.g. Mihailović et al., 1963; Garton et al., 1963; Lasztity, 1966; Pomerantz et al., 1966)

These facts indicated a need for an investigation

into the changes undergone, not only by total lipids, but of lipid fractions during vernalization of cereals.

Two varieties of Secale cereale L., Sangaste fall rye and Prolific spring rye, and 2 varieties of Triticum vulgare L., Kharkov winter wheat and Red Bobs spring wheat, were used. Both rye and wheat were investigated in order to determine whether or not lipid changes varied markedly between the two. Winter and spring varieties were investigated to ensure that lipid changes occurring in the winter varieties would not be wrongly attributed to a vernalization response.

LITERATURE REVIEW

1. Vernalization

In temperate countries the seeds of winter cereals must be planted before the end of winter for the plants to flower and fruit within 12 months of sowing. Spring cereal seeds are sown in the spring and flower and fruit in the summer of the same year.

Early research in this phenomenon was conducted by Klippart (1857). He showed that it was the low temperature of winter that made the winter cereals capable of flowering within a few months after the return of warmer temperatures in spring. Gassner (1918) was the first to attempt an analysis of the low temperature effect. He put forward the idea of a cold requirement in winter cereals not shared by spring cereals. He further demonstrated that the germinating seed is sensitive to the low temperature effect.

Lysenko (1928) established the fact that slight imbibition enables the cereal seeds to respond to the low temperature effect without germinating to an extent which would prevent their being planted by a sowing machine. He coined the word jarovisation for this process which resulted in a winter cereal behaving like a spring cereal. In English, French, German and Netherland translations jarovisation has been translated as vernalization from the Latin vernum meaning spring.

The term vernalization has been given different meanings by different authors. The original restricted meaning was: the substitution of chilling of germinating seed or plants for the natural low temperature of winter, in order to hasten, or to make possible, the initiation of flower primordia at some later date. Chouard (1960) states two derived meanings. First, "any physiological effect of chilling corresponding to the awakening of nature in spring. In this content any effect of chilling that has a forcing effect is called vernalization." Second, "any physiological action stimulating the capacity for flowering, whatever the agent. In this sense vernalization was obtained by heat or by cold, by long days or short days, by light or dark, by nutrition, etc." In this study, the original restricted meaning of vernalization shall be used because it is the most precise and nearest to the initial idea.

2. Conditions Affecting vernalization

The effectiveness of vernalization has been shown to be dependent on several conditions, with some variation according to species or variety. The following may be considered as being applicable to the winter cereals in general.

Low temperature is without effect on dry seeds. Gregory and Purvis (1936), Lojkin (1936), Gregory and De Ropp (1938) and Filippenko (1940) have reported that the

appropriate water content is about 45 to 50% of the dry weight. Lojkin (1936) demonstrated that the response of wheat grains increased with the degree of germination during low temperature treatment, and that a constant moisture content throughout treatment was most effective.

Oxygen was shown by Gregory and Purvis (1938.b) to be absolutely essential for successful vernalization, also necessary are an appropriate duration and temperature of chilling. Gassner (1918) indicated that the lower the temperature used the greater the acceleration of flowering. This, however, though amply substantiated by other workers such as Purvis (1948), Rasumov et al. (1948) and Hansel (1953) has been proved by them to be an incorrect interpretation. It now appears that the response bears a very definite relationship to the duration of low temperature exposure. Temperature level as such has no effect on the response over a fairly wide range. The effective low temperature is between +1 and 6 to 7°. It decreases from 0 to -4° and disappears completely below -6°. It also decreases above +7° and disappears completely between 12 and 14°. Above 15 to 17° the devernializing effect of warm temperatures begins.

The phenomenon of devernialization has been reported in winter rye by Gregory and Purvis (1936), Purvis and Gregory (1952) and others. Purvis and Gregory (1952) have shown that "the degree of devernialization by exposure to subsequent high temperature varies with the initial duration

of vernalization and becomes progressively less as vernalization proceeds." After heat devernalization they succeeded in revernalizing the grain by further exposure to low temperature, and thus established the reversibility of vernalization. They also found that after 12 weeks of vernalization no devernalization occurred in winter rye from subsequent heat treatments. This indicated that the vernalized condition became progressively stabilized with increasing periods of low temperature.

Further studies by Friend and Gregory (1953) indicated that prolonged heating (6 weeks at 20⁰) of partially vernalized winter rye led to an intensification of vernalization, as shown by the acceleration in time to flowering. Such was not the case in spring or fully vernalized winter rye.

The loss of vernalization by drying vernalized grain for periods longer than 6 weeks has been demonstrated by Gregory and Purvis (1938.b) in winter rye. A lack of success attended similar experiments with winter wheat. However, Lojkin (1936) has reported a partial or complete loss of vernalization in fully vernalized wheat seeds kept air dried for 4 weeks at 1 and 15⁰.

Gregory and Purvis (1938.a) applied artificial chilling to winter rye heads varying in maturity from 5 to 35 days after anthesis. They showed that low temperature

was effective from the earliest stage of the embryo and ceased as it became dormant. However, similar experiments carried out on 4 Russian winter wheat varieties failed to indicate successful ear vernalization.

Konovalov (1937), Gregory and Purvis (1938.a), Gregory and De Ropp (1938) and Purvis (1944) have shown that excised embryos when cultivated under aseptic conditions and supplied with sucrose and mineral nutrients were capable of being vernalized. These results indicated that the embryo contains all the prerequisites of vernalization except carbohydrates and oxygen.

Gott et al. (1955) reported that in both the winter and spring forms of Petkus rye maximum earliness is attained in continuous light. In both flowering fails when they are grown continuously in days of less than 11 to 12 hours duration. Dissection, however, reveals that ears are differentiated under such conditions but die without emerging from the leaf sheaths. Short days then do not inhibit the inception of flowering, but later stages, including the extension of internodes, require long days.

3. The Vernalization Process

There are essentially 3 schools of thought concerning the nature of the vernalization reaction.

The first favours the view that any modification

of the protoplasm of the plant that may be associated with the transition to flowering are induced by the formation and action of hormone - like substances.

The second suggests that biochemical changes occurring in the protoplasm during the vernalization process are responsible for the induction of early flowering in winter varieties.

The third considers that the early flowering of winter varieties resulting from vernalization is due to biophysical changes occurring in the protoplasm during low temperature treatment.

These 3 views will now be discussed in turn.

a. Hormonal Theory

At present the most tangible evidence existing for the involvement of a definite substance in vernalization is that the vernalized condition in Hyoscyamus was shown to be transmissible through a graft union by Melchers (1939). This experiment, however, has only been performed once, and for this reason Melchers (1952) himself questions its validity.

Attempts to isolate flower inducing substances, or even to obtain active extracts from vernalized plants are widespread in literature and the results are controversial.

Melchers and Lang (1941) report that careful

experimentation has failed to extract any florigenic substance from Hyoscyamus. A later report by Napp-Zinn (1956) bears this out. Purvis and Gregory (1953) have, however, on several occasions obtained small accelerations to heading by adding a chloroform extract of vernalized rye embryos to the substratum on which unvernallized embryos were germinated. These small positive effects were not statistically significant. Further experiments with greatly increased replication while repeating the differential effect failed to increase the significance. These workers also used water, ethyl alcohol, and ether extracts without success. In contrast to this Highkin (1955) has demonstrated that if the water used to soak pea seeds for 25 days at 4° was then used to soak other pea seeds which were immediately planted without chilling, it caused a significant reduction in the age of the appearance of the first flower. He further reported that this soak water also induced early flowering in other vernalization requiring plants such as winter cereals.

Tomita (1958, 1959.a, 1959 .b, 1961, 1962.a, 1962.b, 1963.a, 1963.b) has carried out a number of experiments on flower induction of winter rye, winter wheat, annual blue grass, and radish. He suggests that the flowering factor may be a nucleic acid derivative. Diffusate of vernalized winter rye seeds which induces flowering in unvernallized winter rye, unvernallized winter wheat, and annual blue grass contains unidentified auxins, free amino acids and nucleotides. One of the nucleotides that is most

effective in floral promotion was identified by paper chromatography and electrophoresis as uridylic acid. Authentic uridylic acid was found by Tomita (1961, 1963.b) to promote flowering in unvernallized winter wheat. Devernallization could be attributed to the heat instability of the nucleotide. Tomita (1962.b, 1963.b) also demonstrated that the water diffusate and juice from vernalized radish promote flowering in unvernallized radish.

Oota (1964) has reported that Yamashita et al. showed that RNA isolated by Schramm's phenol method from vernalized radish seeds as well as from photoperiodically induced leaves of morning glory caused an enhancement and induction of floral buds in Lemna paucicostata. The phenol extracted RNAs of vernalized and unvernallized radish seeds were shown to have different guanylic and uridylic acid contents.

In contrast to the above findings however, Sarkar (1958) reported that he had failed to obtain any effect of nucleic acid constituents on the flowering response in vernalized and unvernallized Hyoscyamus plants

It is of interest to note that the above effects are characterized by their marked non-histospecificity. This non-specific nature of the stimulus was demonstrated in Melchers (1939) experiments. He showed that leafy stems or even single leaves from annual plants or from vernalized biennial plants of Hyoscyamus when grafted on unvernallized

stocks in the neighbourhood of the growing point conferred on them the ability to flower. This ability it was also found, could be conferred by scions of other species.

That macromolecules such as nucleic acids could cause an effect when applied externally can be explained by the findings of Ledoux (1963), Ledoux and Galand (1963) and numerous others detailed by Ledoux (1965). It has been shown using the seed of germinating barley that ^3H or ^{14}C -labelled macromolecules can be transferred intact from the seed to different organs. In addition exogenous nucleic acids and proteins can penetrate cell membranes and become deposited in tissues in macromolecular form.

There is considerable evidence that nucleic acid metabolism is implicated in the ultimate control of flowering. This has been revealed by Cutter (1965). It is not surprising therefore, that numerous investigations have been carried out on the effect of various nucleic acid antimetabolites on plant growth and flowering. The close relationship of proteins and nucleic acids has resulted in similar studies being carried out with protein antimetabolites.

Evans (1964) reported an inhibition of flower induction on application of actinomycin D to Lolium temulentum. Earlier work by Slotnick (1960) has shown this antibiotic to be a potent inhibitor of growth in bacteria. Its action has been attributed by Kirk (1960) to specifically inhibiting DNA dependent RNA synthesis through binding to the guanine

residues of DNA. Later work by Kahan et al. (1963) showed that the antibiotic binds to DNA and prevents the synthesis of RNA by DNA dependent polymerase. In fact at high concentrations the synthesis of DNA is inhibited as well (Hurwitz et al., 1962). These findings were extended by Jacob and Monod (1961) who stated that the blocking of the synthesis of messenger RNA presumably stops the synthesis of proteins which in turn leads to the inhibition of growth. On the other hand Del Valle and Aronson (1962) suggest that some developmental processes are not affected by actinomycin D, or there seems to be a definite lag in its action, at least as far as protein synthesis is concerned, as suggested by Gross and Cousineau (1963). This apparent lag in the action of actinomycin D is demonstrated by the work of Bal and Gross (1963) which showed that in plants a high concentration of actinomycin D inhibited mitosis in the root meristem after a delay of 36 hours and subsequently the synthesis of RNA.

Chloramphenicol has been found by Rabson and Novelli (1960) and Margulies (1962) to be a specific inhibitor of protein synthesis when applied to plants in high concentration. Kruzhilin and Shvedskaya (1963) reported that this antimetabolite inhibited the development of wheat plants and the synthesis of amino acids at the end of vernalization. Dévay (1965) reported that chloramphenicol inhibition was evidenced primarily during the middle phase of vernalization. From her results it was concluded that during vernalization

specific RNase synthesis takes place at the beginning of cold induction probably followed by the synthesis of specific proteins.

Evans (1964) injected chloramphenicol into the apical area of Lolium temulentum and found it to be inhibitory to flowering. On the other hand Collins et al. (1963) did not find it to inhibit flowering when applied to the leaf and buds of Cocklebur plants. In contrast to the above findings Feierabend et al. (1967) have reported that when winter rye seedlings were incompletely vernalized, flowering could be promoted by treatment with 0.05% chloramphenicol during the chilling period.

Bonner, Salisbury and Zeevaart (1960, 1962) have shown with Xanthium, and Evans (1964) has shown with Lolium temulentum that inhibition of flowering by 5 - fluorouracil correlates with inhibition of synthesis of RNA in the apex at a critical time during which the floral stimulus from photoperiodic induction is present. The 5 - fluorouracil inhibition was reversed by the RNA precursor orotic acid but not by the DNA metabolite thymidine. Further experiments by Zeevaart (1962) with the inhibitor 5 - fluorodeoxyuridine demonstrated blocking of flowering in Pharbitis nil along with the simultaneous arrest of DNA multiplication and cell division in the bud. Harbers et al. (1959), Hartman and Heidelberger (1961), Taylor et al. (1962) and Kihlman (1963) have shown that 5 - fluorodeoxyuridine inhibits the synthesis of DNA by blocking the formation of thymidylic

acid. This effect could be reversed by post treatment with thymidine. The effects of both the pyrimidine antimetabolites were temporary. The buds were once more inducible when the inhibitors had dissipated and normal cell division had resumed.

Numerous attempts have been made to hasten the flowering by treatment of seeds or plants with growth hormones. The 2 hormones most commonly used are auxin and gibberellin. These studies have yielded controversial results.

Cholodny (1935) detected a large supply of an auxin - like hormone in the endosperm of maize and other cereals. This he named blastenin, and suggested that at normal temperatures this is transferred to the embryo and immediately used for extension growth. At low temperature, however, retarded growth increases the concentration of blastenin in the meristem which, Cholodny (1936, 1939) states accelerates developmental processes in the cells before they undergo much growth. Recently Brugovitsky and Bosica (1967) reported a similar finding of stimulatory substances of the indole type during the vernalization of winter wheat. The hypothesis of Cholodny (1935) has been suggested as being quite untenable. Hatcher (1945) demonstrated the complete absence of auxin from the vernalized embryos of rye. Further, as has been already stated, it is possible to vernalize embryos deprived of endosperm and provided with only mineral salts and sugar. Thus if growth hormones are needed for the vernalization reaction they can be supplied

by the embryos themselves.

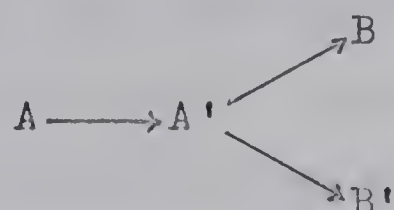
Melchers (1936, 1937) reported that in his experiments with Hyoscyamus he had found it impossible to induce flowering in biennial stock in its first year by applying auxin instead of a scion from an annual or vernalized plant. On the other hand Thimann and Lane (1938) stated that they had obtained an acceleration of flowering by soaking various seeds with synthetic auxins.

Leopold and Thimann (1949) increased the auxin content of Wintex barley by a leaf injection method. They record that the number of flowers was increased at an optimal concentration, and reduced as the concentration was further increased. Hussey and Gregory (1954), however, while confirming this for Wintex barley, obtained no such effect in winter rye. They attributed the effect to acceleration of apical growth rather than of development. These studies were extended by Leopold and Guernsey (1953). They showed that the result of treating seeds (Biloxi soy bean, Wintex barley, Victory oats and sweet corn) may be either positive or negative according to the temperature following the hormone treatment. A period (10 to 14) days at a vernalizing temperature induces a positive response in auxin treated seeds which increases with the concentration of auxin to a maximum and then declines. At room temperature the same concentration of auxin consistently inhibits.

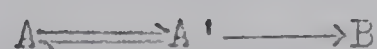
Gibberellin application to Hyoscyamus has been shown by Melchers (1937) to produce a response differing from that to normal vernalization. After treatment with gibberellin the first flower primordia arise directly in the rosette stage. This is supported by Sarkar (1958) who found a similar occurrence in Arabidopsis thaliana as a result of gibberellin treatment. However, Sarkar further reports that he was able to produce a similar effect by low temperature treatment. What is interesting is that Arabidopsis seeds, which respond to vernalization, did not respond to gibberellin treatment. This supports the findings of Lona and Bocchi (1956) who record a failure to replace vernalization treatment by the application of gibberellin to winter wheat and rye seeds. Caso et al. (1960) and Rejowski (1965) also substantiate this with winter rye and winter wheat. They reported that gibberellin application only resulted in an acceleration in flowering following treatment of the growing plant. Their findings differ however, in that Caso et al. and Purvis report that they obtained a response by treating unvernallized winter rye plants provided several leaves were present. Rejowski was unable to obtain a similar response with winter wheat unless it had been vernalized for 15 days. Lang et al. (1957) and Chailakhyan (1966) have reported that there is natural occurrence of gibberellin like substances in the tissues of higher plants.

The present situation is that although there has been much experimental work by supporters of the hormone theory there has as yet been no isolation of a flower inducing substance. Therefore, the hypothetical schemes of Lang and Melchers (1947) and Purvis and Gregory (1952) remain still to be proven or disproven. These schemes which have been proposed for the mechanism of the vernalization process may be formulated as follows.

Lang and Melchers



Purvis and Gregory



A is a precursor, A' is a labile product and B is the stable end product of vernalization.

The schemes differ in only one point. Lang and Melchers assume that A' is subject to two competing processes, one leading to B, the other to another product B' which is inactive with respect to flowering. Purvis and Gregory assume that the process $A \rightleftharpoons A'$ proceeds at low temperatures predominantly from left to right, but vice versa at higher temperatures. At higher temperatures, therefore, there would be no net production of A', B would either accumulate very slowly or not at all with a consequent delay or failure of flowering.

b. Biochemical Changes Theory

A great many investigations have been devoted to this question. The investigations consist essentially in comparing treated tissue during and at the end of vernalization with non chilled tissue. The results serve to check on the completeness and effectiveness of the vernalizing process or to try to observe the precise nature of the process.

Several papers report that carbohydrates are depolymerized resulting in an increase of soluble sugars in winter varieties during cold treatment (Aksenova, 1960; Valuta and Brad, 1960; Sparmann, 1961; Pavlov and Tyankova, 1962; Devay, 1962; Kruzhilin and Shvedskaya, 1963; Shvedskaya and Kruzhilin, 1964; Trione, 1966). Some papers indicate no difference between the soluble sugar content of spring and winter cereal varieties grown in the cold (Grzesiuk and Kulka, 1962; Moskov and Bozova, 1962). Others (Kruzhilin, 1963; Sechet, 1962.b; Trione, 1966) indicate that the winter varieties accumulate more soluble sugars than spring varieties when both are grown in a cold environment. In this connection Dévay (1962) reports that temperature curves of amylase and maltase activity show a dependence on the degree of winter habit. She says that it can be observed generally that enzyme activity in winter varieties is observable at 0° while they respond to higher temperatures with a reduction of activity. In summer varieties on the other hand, amylase and maltase activities

can be shown practically above 10° only. The poor temperature response of summer varieties can probably be explained by the low activity of their enzymes at low temperature.

Reserve proteins are depolymerized during the low temperature treatment resulting in an increase in soluble nitrogen compounds (Sparmann, 1961; Kruzhilin and Shvedskaya, 1963). In this connection Trione (1966) reports that the soluble nitrogen fraction of winter wheat grown at low temperature rose nearly threefold as compared to that of spring wheat grown under similar conditions. On the other hand there was practically no difference between the soluble protein fraction of the two when they were both grown at 25°. Recent work by Teraoka (1967) has indicated that during vernalization of wheat embryos new proteins are detectable. However, after vernalization the protein patterns of winter wheat embryos were very similar to that of spring wheat embryos.

David (1947, 1951) working with wheat, and Valuta and Brad (1958) working with rye have reported a decrease in total lipids during the first 4 weeks of vernalization, after which they again increased. On the other hand Duperon (1949, 1952) working with mustard, radish and oats observed a continuous decrease in total lipids during vernalization. This last finding is not surprising.

In seeds such as these with a high lipid content lipids form one of the sources of food material during germination.

Organic acids have also been observed to change during vernalization. Kruzhilin and Shvedskaya (1963) reported that in wheat during vernalization there was, during the first three weeks, an increase of oxalic and tartaric acids in the endosperm, and aconitic, malic, and α -ketoglutaric in the germ. After this period pyruvic acid increased. These workers in the following year (1964) reported that in cabbage buds there was in the second half of vernalization a disappearance of dicarboxylic acids and accumulation of citric acid. Ascorbic acid has been observed by Michniewicz (1961) and Telcherova (1964) to increase during vernalization. Michniewicz further reported that the seed after vernalization was higher in ascorbic acid than the unvernallized seed at the same stage of growth.

Dé'vay (1964) reports an increase in ascorbinoxidase activity in wheat during vernalization. She states that as a consequence of the increase of ascorbic acid oxidase activity there is an increase in dehydroascorbic acid. This dehydroascorbic acid as a cofactor regulates the SH group content which also shows an increase. This gains support from Telcherova (1964) who has reported an increase of SH group content during vernalization of wheat varieties.

Finch and Carr (1956) reported that they were unable to find any differences in the contents of DNA and RNA in Petkus winter rye embryos in relation to vernalization. This finding has not met with general agreement however. Konarev (1954) and Teraoka et al. as reported by Oota (1964) have stated that low temperature treatment definitely elevates the total RNA level in winter wheat embryos. Konarev goes on to report that the difference was greater in winter than summer varieties. Sechet (1962 a. b.) in addition to an increase in RNA also says that there is an increase in DNA. On the other hand Stoletov et al. (1965) state that vernalization generally produced a decrease in the nucleic acid content. Still another view is presented by Hayashi and Nagata (1965). They demonstrated that at the initiation of vernalization the nucleic acid content of the seed of vernalizing Japanese radish decreased, reached a minimum on the fifth day of low temperature treatment, and thereafter increased again.

c. Biophysical Changes Theory

This theory has few supporters. A number of early Russian workers attempting to justify Lysenko's hypothesis of phasic development (discussed in full in reviews by Maximov, 1934; Murneek and Whyte, 1948; Whyte 1949) produced evidence that low temperature changes the physical condition of the protoplasm. For example Richter (1934) demonstrated a shifting of the isoelectric point;

this was gradual and increased with the duration of exposure to cold. Čajlachjan (1935) and Filippenko (1936) noted increased permeability after vernalization in winter wheat. Čajlachjan suggests that this is due to a change in the structure of proteins of winter wheat as a result of which the structure becomes typical for spring wheat. Bassarskaya (1936) reports that low temperature altered staining reactions and plasmolytic responses.

Section 1. Testing of Method for Effecting Vernalization

Materials and Methods

Sangaste fall rye was used for this study. The 11 treatments employed are detailed in Table 1.

The seeds were germinated on moist filter paper in plastic dishes. The sequence of soaking and germination was such that all the cold treatments commenced simultaneously.

Ten ml of distilled water were added to all dishes except those containing the seeds which were to serve as cold controls. The dishes were covered with parafilm in which 4 holes had been punched to permit aeration. They were placed in a cold room maintained at 2 to 4° in continuous illumination of 3 ft - c. During cold treatment the moisture content of the dishes was maintained at a constant level by regular weighings and additions of water.

On termination of cold treatment the seeds were planted in a growth chamber in 15 cm diameter plastic pots, at the rate of 4 seedlings per pot. The pots were arranged in 3 blocks of a randomized block design.

The potting mixture consisted of fine sand and peat moss 1 : 1 supplemented with a fertilizer mixture containing potassium nitrate, potassium sulfate, superphosphate (18 to 20% P_2O_5), calcium and magnesium carbonate.

Table 1. Number of days from planting to heading of Sangaste fall rye as affected by soaking and/or germination of seeds prior to vernalization, and by duration of vernalization.

<u>Treatment</u>			<u>Number of days from planting to heading</u>
Unvernalized control			139
Dry seeds kept at $3^{\circ} \pm 1^{\circ}$ for 6 weeks			139
Dry seeds kept at $3^{\circ} \pm 1^{\circ}$ for 10 weeks			139
<u>Hours soaking</u>	<u>Hours germination</u>	<u>Weeks vernalization</u>	
6	24	6	38
0	24	6	38
6	0	6	42
0	0	6	41
6	24	10	28
0	24	10	29
6	0	10	29
0	0	10	30

The data are an average of 3 replicates with 4 plants per replicate.

The conditions in the growth chamber were, 15⁰, 1700 ft - c, continuous light and 60% relative humidity.

Number of days from planting to heading were recorded for each plant.

Results and Discussion

The results given in Table 1 showed that:-

Low temperature was without effect on dry seeds. Seeds kept at 2 to 4⁰ for 6 and 10 weeks produced plants which headed at the same time as the unvernallized controls. Gregory and Purvis (1936), Lojkin (1936) and Filippenko (1940) have all reported a similar finding.

The number of days from planting to heading was not markedly affected by soaking and/or germination of seeds prior to vernalization. This can probably be explained by the fact that during vernalization available water was not a limiting factor. The seeds were therefore able to germinate as rapidly as the low temperature would permit. This would result, as has been demonstrated by Lojkin (1936), in a maximum response to vernalization which would tend to remove any advantage gained by the seeds which had been presoaked and/or pregerminated.

The number of days from planting to heading was shortened by increasing the duration of low temperature treatment. In all cases, plants produced by seeds vernalized



FIG 1. Photograph showing vernalized and unvernallized Sangaste fall rye plants.
1. Vernalized Sangaste fall rye plants.
2. Unvernallized Sangaste fall rye plants.

for 10 weeks headed in a much shorter time than plants produced by seeds vernalized for 6 weeks. Purvis (1948), Rasumov et al. (1948) and H^ánsel (1953) obtained similar results.

It should be noted, that if the 70 days of cold treatment required for most effective vernalization, is added to the 28 days required for the most effectively vernalized seedlings to attain heading, the total of 98 days is still much shorter than the time required for the unvernallized plants to head.

As may be seen from Fig 1 the vernalized plants had headed while the unvernallized plants were still in the rosette stage.

These results indicated that the method described above was suitable for effecting vernalization.

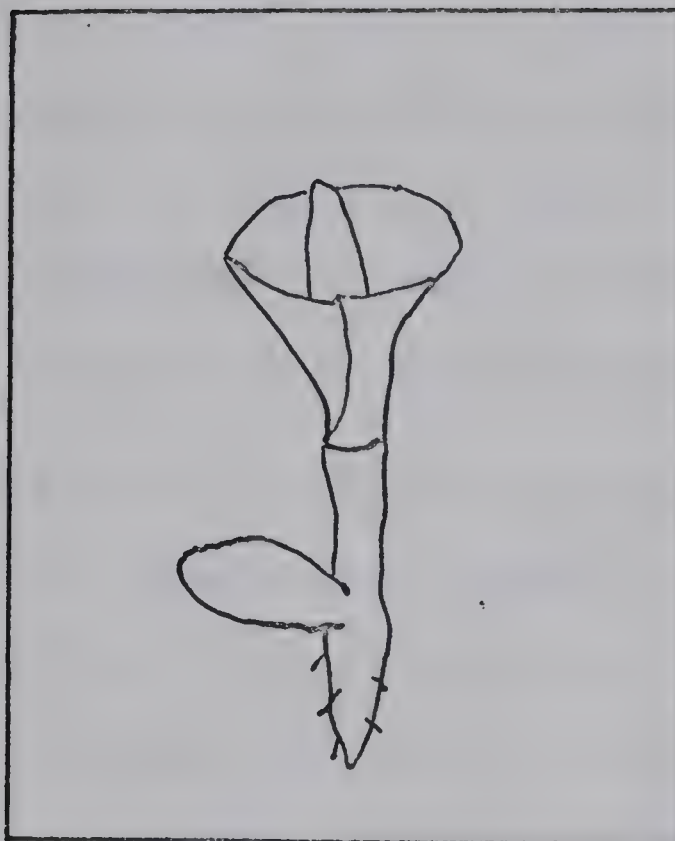


FIG 2. Diagram showing a parafilm funnel fitted on the coleoptile of a seedling.

Section 11. Effects of Vernalized Tissue Extracts and
Nucleic Acids on Unvernalized Material

Materials and Methods

A few hundred grams of Sangaste fall rye seeds were divided among several plastic dishes containing filter paper which had been moistened with distilled water. These dishes were covered with parafilm in the center of which a hole was cut to facilitate aeration and addition of water.

Following a 24 hour germination period at room temperature, the dishes were placed in a cold room maintained at 2 to 4° under continuous illumination of 3 ft - c. During cold treatment the moisture content of the dishes was maintained at a constant level by regular weighings and additions of distilled water.

a. Vernalized Tissue Extracts

After 10 weeks of cold treatment, several of the vernalized seedlings together with unvernalized seedlings grown at room temperature to a coleoptile length of 1 cm, were planted in a growth chamber as described in Section 1. All the seedlings except the vernalized and unvernalized controls were fitted with parafilm funnels as shown in Fig 2.

Two batches of 50 vernalized seedlings were crushed thoroughly in a chilled mortar with 100 ml of phosphate buffer 0.1 M pH 7.0, or deionized water pH 6.2. A small

quantity of fine sand was added as an abrasive. The resulting mixture was filtered through 4 layers of cheesecloth, and further clarified by centrifugation for 30 minutes at 4° at 1500 x g.

One half the pots in the growth chamber containing unvernallized seedlings, designated to receive applications of vernalized seedling extract or control solutions of phosphate buffer or deionized water, were removed to the cold room. To these, and to the corresponding seedlings in the growth chamber, the solutions were applied by addition to the parafilm funnels at the rate of 1 ml per plant. The seedlings receiving cold room applications of solutions were allowed to remain at 2 to 4° for 3 days prior to being replaced in the growth chamber. Seven days later a further application of freshly prepared solution was made under similar conditions. The treatments are indicated in Table 11.a.

The final number of days from planting to heading were recorded for each plant.

b. Nucleic Acids

1. Ribonucleic Acid

The extraction procedure was similar to that described by Laskov et al. (1959).

One hundred grams of vernalized seedlings were

placed in a stainless steel press precooled to 4° . After covering with 40 ml of a 1 : 1 mixture of 10^{-4} M ethylenediaminetetraacetate (EDTA) adjusted to pH 8.0 and 90% w/v freshly redistilled phenol solution, the tissue was crushed under a pressure of 1400 kg per sq cm through a stainless steel sieve with holes 1 mm in diameter. A further 80 ml of solution were added to the resultant slurry. This slurry was then gently stirred for 1 hour at room temperature and centrifuged for 3 minutes at $10,000 \times g$ at 4° . The cloudy top aqueous solution was carefully removed without disturbing the interface. The remaining phenol phase was extracted once with 200 ml 10^{-4} M EDTA pH 8.0. The aqueous extracts were combined and centrifuged for 20 minutes at $10,000 \times g$. The RNA was precipitated by the method described by Littauer and Eisenberg (1959). Two volumes of cold 95% ethanol containing 2% of potassium acetate were added to the cold aqueous solution. After 30 minutes at 4° the precipitate was removed by centrifugation for 10 minutes at $10,000 \times g$. The precipitate was washed with cold 75% ethanol containing 2% of potassium acetate and then with cold 75% ethanol. The precipitate was dissolved in 10 ml of tris buffer 0.01 M pH 7.4. The resulting solution was cleared by centrifugation for 20 minutes at $10,000 \times g$. The RNA was utilized immediately.

Samples of RNA prepared as described above gave a clear solution with a typical RNA spectrum, which

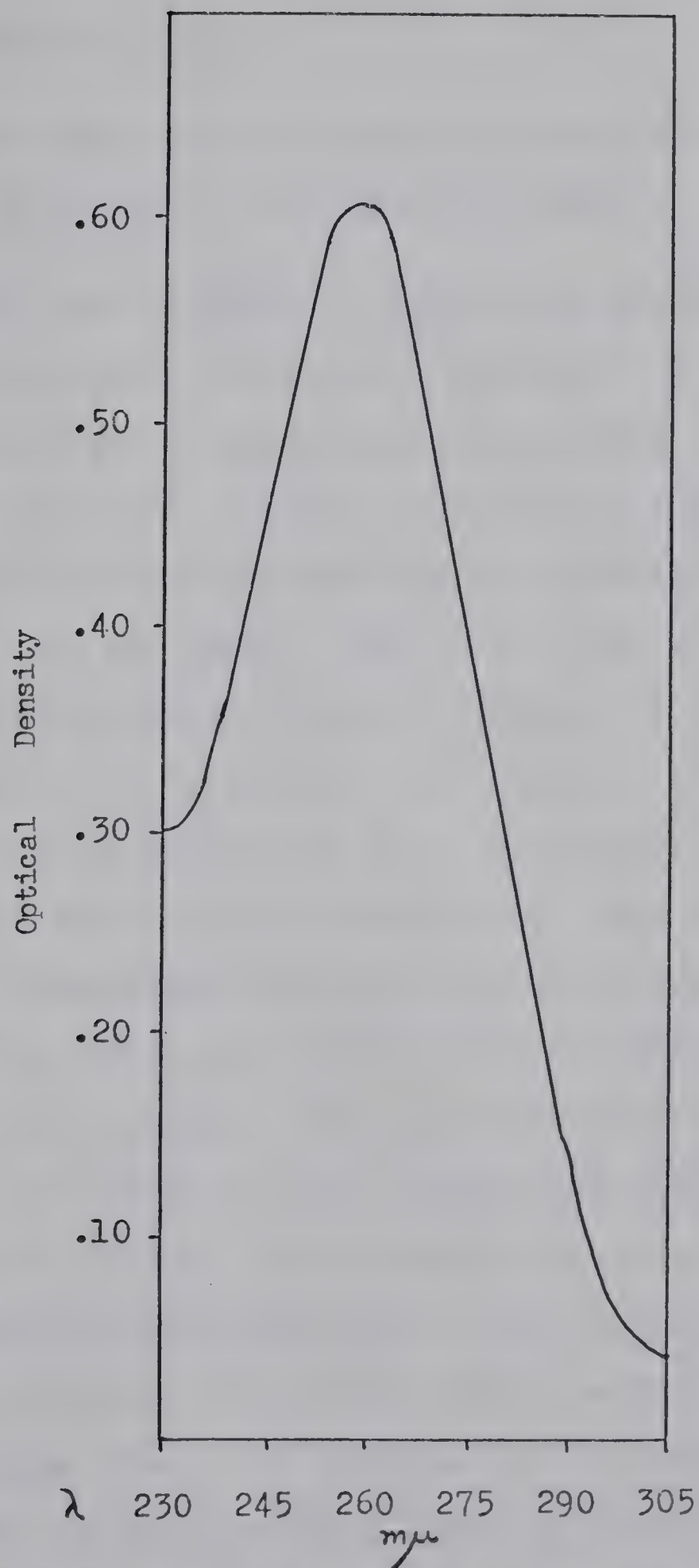


FIG 3. Spectrum of RNA isolated from vernalized Sangaste fall rye seedlings by the methods of Laskov et al. (1959) and Littauer and Eisenberg (1959).

exhibited an absorption maximum at 259 m μ in a Beckman DK - 1 spectrophotometer (Fig 3).

2. Deoxyribonucleic Acid

The extraction procedure was similar to that carried out by Lyttleton and Petersen (1964).

One hundred grams of vernalized seedlings were placed in a stainless steel press precooled to 4⁰. After covering with 40 ml of sodium chloride - tris - EDTA buffer pH 7.5, the tissue was crushed as described for RNA extraction. The insoluble material was removed by filtration through Whatman No. 3 filter paper. The first 25 ml of filtrate were refiltered to obtain better retention of the insoluble material. When the filtration was complete, the insoluble residue was washed twice with 30 ml of buffer. The filter paper carrying the insoluble residue was then briefly (30 seconds) dispersed with 150 ml of 1% sodium dodecylsulfate in 0.025 M tris buffer pH 7.3 in a Servall Omnimixer at one - third line voltage. It was transferred to a flask and stirred for 1 hour at room temperature with a volume of phenol equal to that of the extraction solution. The phases were separated by centrifugation at 1000 x g for 10 minutes. The aqueous phase was removed by gentle suction and the DNA was precipitated from it by addition of 2 volumes of 95% ethanol. The precipitate was allowed to settle for 1 hour in the cold, spun off, and redissolved in 10 ml 0.015 M sodium citrate, 0.15 M sodium chloride (citrate saline).

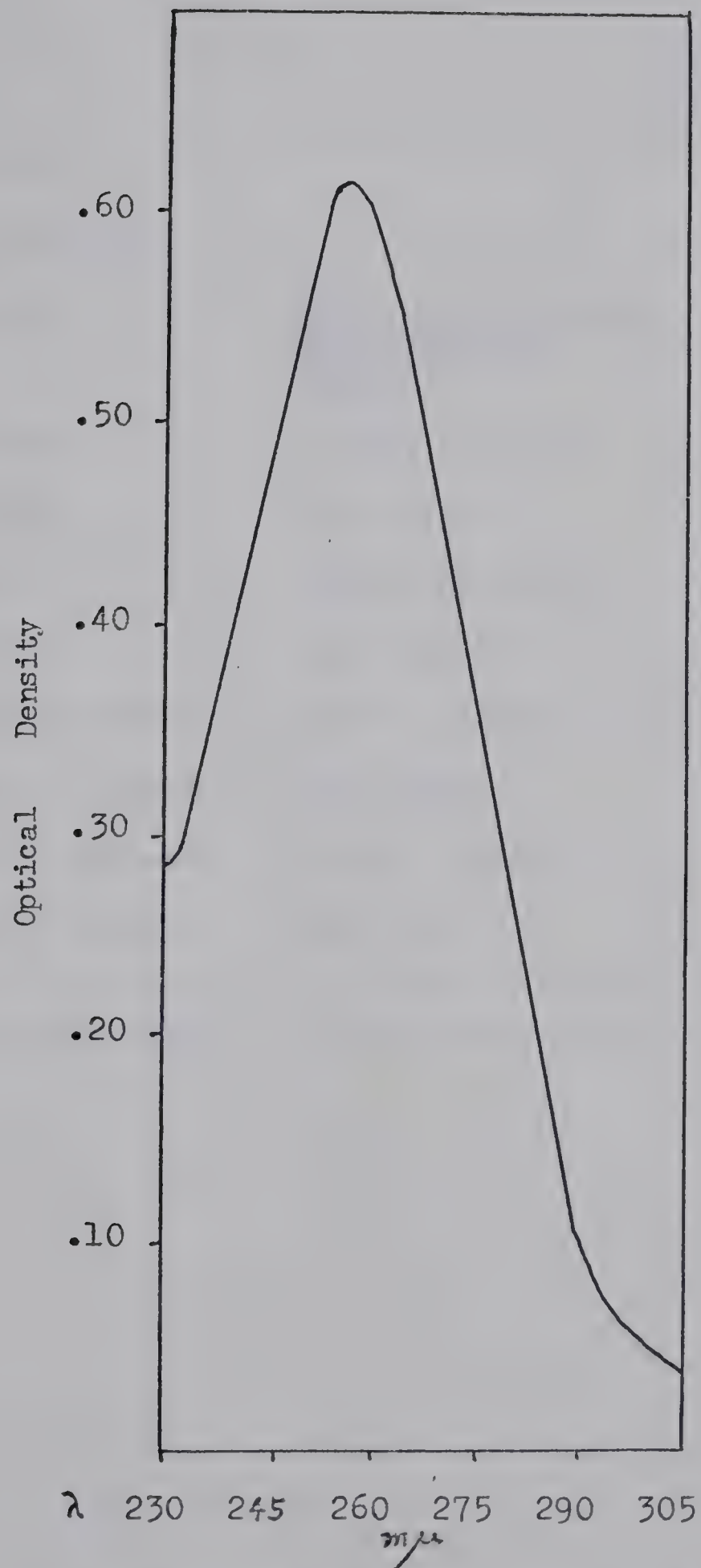


FIG 4. Spectrum of DNA isolated from vernalized Sangaste fall rye seedlings by the method of Lyttleton and Petersen (1964).

Table 11.a. Number of days from planting to heading of unvernallized Sangaste fall rye as affected by applications of vernalized plant extracts.

<u>Treatment</u>		<u>Number of days from planting to heading</u>
Vernalized control		31
Unvernallized control		140
<u>Chemical applied</u>	<u>Location of plants when chemical applied</u>	
Phosphate buffer	Growth chamber	136
Phosphate buffer	Cold room	139
Deionized water	Growth chamber	143
Deionized water	Cold room	141
Phosphate buffer extract	Growth chamber	138
Phosphate buffer extract	Cold room	137
Deionized water extract	Growth chamber	140
Deionized water extract	Cold room	141

The data are an average of 3 replicates with 4 plants per replicate.

Table 11.b. Number of days from planting to heading of unvernallized Sangaste fall rye as affected by applications of nucleic acids.

<u>Treatment</u>		<u>Number of days from planting to heading</u>
Tris buffer control		141
<u>Hours of germination</u>	<u>ml of RNA solution applied</u>	
0	1	143
0	5	139
0	10	141
8	1	136
8	5	138
8	10	137
24	1	143
24	5	142
24	10	141
Citrate saline control		140
<u>Hours of germination</u>	<u>ml of DNA solution applied</u>	
0	1	140
0	2	139
0	5	142
8	1	136
8	2	138
8	5	137
24	1	140
24	2	140
24	5	138

The data are an average of 3 replicates with 4 plants per replicate.

This solution was clarified at 20,000 x g for 15 minutes, and an equal volume of 2 M sodium chloride was added to the supernatant. After 18 hours at 4° this solution was centrifuged at 10,000 x g for 20 minutes and the DNA precipitated from the supernatant with 2 volumes of 95% ethanol. The fibres which formed were immediately withdrawn by spooling on a glass rod, pressed dry, and redissolved in 5 ml of citrate saline. This DNA was utilized immediately.

Samples of DNA prepared as described above gave a clear solution with an absorbance maximum at 258 m μ in a Beckman DK - 1 spectrophotometer (Fig 4). This absorption maximum corresponds to that given in the literature.

Three concentrations of the RNA in tris buffer, and 3 concentrations of the DNA in citrate saline, along with the appropriate controls, were applied to seeds which had been germinated for 0, 8, and 24 hours (Table 11.b). The seeds were germinated in petri dishes containing deionized water. They were soaked overnight in the solutions and were then planted in a growth chamber as described in Section 1.

Number of days from planting to heading were recorded for each plant.

Results and Discussion

The results summarized in Tables 11.a and 11.b show that the applied treatments had no apparent effect. All

plants grew normally and headed between 137 and 143 days which was the usual length of time to heading of unvernallized Sangaste fall rye under the conditions of the study.

There are several possible causes for failure of the vernalized tissue extracts and nucleic acids to accelerate flowering when applied to unvernallized material.

The solvents used may not have extracted the so called active vernalization product or may have inactivated it. It is possible that the isolation procedure inactivates the elusive compound.

The substance may not have been absorbed by the test plants, or at least not in sufficient quantity to produce an effect. Ledoux (1965) reported that a technique to ensure uptake of DNA by germinating barley consisted in sectioning the seed at one end and standing it in a drop of test solution. However, employing this method in a number of preliminary experiments extensive fungal growth occurred, hence, this method was not used.

Another possibility is that there is in fact no active substance. Wellensiek (1962) in this connection has put forward very convincing arguments for the view that one universal theory of flowering is not conceivable. He states, "we find a great number of very different mechanisms which all result in flowering. It seems to be

rather improbable that one and the same substance, i.e. the flowering hormone, could be synthesized in so many ways."

Section 111. Effects of Protein and Nucleic Acid Antimetabolites
on Vernalizing and Vernalized Seedlings

Materials and Methods

Sangaste fall rye was used in this study. Fourteen lots of 20 seeds each, and 1 lot of 200 seeds, were placed in plastic dishes containing filter paper overlying vermiculite which had been moistened with distilled water. In those dishes containing the 20 seed lots each seed was placed in a hole in the filter paper with the radicle end pointing downward. This maintained the seeds in constant positions and prevented bending of the coleoptiles. Aeration and addition of water was facilitated by means of a hole cut in the center of the filter paper and a corresponding hole cut in the parafilm with which the dishes were covered.

Following a 24 hour germination period at room temperature, the dishes were placed in a cold room maintained at 2 to 4° under continuous illumination of 3 ft - c. During cold treatment the moisture content of the dishes was kept constant by regular weighings and additions of distilled water.

After 1 week in the cold room the coleoptiles had attained an average height of 1 cm. Parafilm funnels were now placed on the coleoptiles of the seedlings (Fig 1) in those dishes containing the 20 seed lots. A period of 3 days was allowed for the coleoptiles to adjust to the funnels

and to test for leakage. Following this a total of 9 applications of antimetabolite or control solution (Table 111.a) were applied by adding 0.1 ml of solution to each funnel every 7 days.

Preliminary trials indicated that the highest concentrations of the antimetabolites were sufficient to seriously retard or completely inhibit germination.

In preparation of the antimetabolites, actinomycin D and chloramphenicol, which are only slightly soluble in water, were first dissolved in 95% ethanol and then distilled water added to final volume (alcohol concentration 50%).

5 - Fluorouracil and 5 - fluorodeoxyuridine were dissolved in distilled water.

After 10 weeks of treatment the funnels were removed. These seedlings, together with vernalized seedlings from the 200 seed lot and unvernallized seeds, were planted in a growth chamber as described in Section 1. With the exception of the controls, all the vernalized seedlings from the 200 seed lot were fitted with parafilm funnels. An antimetabolite or control solution (Table 111.b) was now applied as described above. Treatment had to be discontinued after 3 applications due to rupturing of the funnels by seedling growth.

During growth, weekly countings were made of the number of tillers per plant. Any deviations from normal

Table 111. Effect of protein and nucleic acid antimetabolites on number of days from planting to heading of Sangaste fall rye when applied during and after vernalization.

<u>Treatment</u>	<u>Number of days from planting to heading</u>	<u>Treatment</u>	<u>Number of days from planting to heading</u>
<u>a. Plants undergoing vernalization were treated 9 times with 0.1 ml containing</u>		<u>b. Vernalized control 31bcd</u> <u>Unvernalized control 140e</u>	
50% ethanol water	32bcd 31bcd	<u>Vernalized plants were treated 3 times with 0.1 ml containing</u>	
<u>actinomycin D</u>		50% ethanol water	32bcd 31bcd
50.00 µg	30cd	<u>actinomycin D</u>	
12.50 µg	30cd	50.00 µg	32bcd
6.25 µg	29d	12.50 µg	33bcd
<u>chloramphenicol</u>		6.25 µg	32bcd
1.50 mg	34abcd	<u>chloramphenicol</u>	
1.00 mg	31bcd	1.50 mg	35abc
0.50 mg	32bcd	1.00 mg	35abc
<u>5 - fluorouracil</u>		0.50 mg	35abc
130.0 µg	39a	<u>5 - fluorouracil</u>	
13.0 µg	33bcd	130.0 µg	31bcd
1.3 µg	31bcd	13.0 µg	32bcd
<u>5 - fluorodeoxyuridine</u>		1.3 µg	33bcd
24.5 µg	36ab	<u>5 - fluorodeoxyuridine</u>	
2.45 µg	32bcd	24.5 µg	34abcd
0.245 µg	31bcd	2.45 µg	32bcd
		0.245 µg	30cd

Numbers that are not followed by the same letter are significantly different from each other at the 5% level of significance as judged by Duncan's Multiple Range Test (1955).

The data are an average of 3 replicates with 4 plants per replicate.

Table 1V. Analysis of variance for the data concerning the effect of protein and nucleic acid antimetabolites on number of days from planting to heading of Sangaste fall rye when applied during and after vernalization

		<u>using vernalized</u>		<u>using unvernallized</u>	
		<u>plants as controls.</u>		<u>plants as controls.</u>	
<u>Source</u>	<u>df</u>	<u>ms</u>	<u>F</u>	<u>ms</u>	<u>F</u>
Treatments	28	13.6	2.8**	1294.2	269.6**
Error	56	4.8		4.8	

** Significant at the 1% level.

growth were noted, and the final number of days from planting to heading was recorded for each plant.

Results and Discussion

An analysis of variance (Table IV) for the data summarized in Table III shows that when the vernalized plants were used as controls there was a significant difference at the 1% level between treatments. Duncan's Multiple Range Test (1955) was therefore used to determine which of the treatments were significantly different. The results revealed that although significant differences did exist between various treatments no overall pattern could be distinguished. Applications of 5 - fluorouracil ($130.0 \mu\text{g}/0.1 \text{ ml}$) to plants undergoing vernalization appeared to be the most effective in delaying flowering. It was in fact the only treatment that differed significantly from the vernalized control. Actinomycin D ($6.25 \mu\text{g}/0.1 \text{ ml}$) applied to plants undergoing vernalization was the least effective in delaying flowering but differed significantly only from the 5 - fluorodeoxyuridine ($24.5 \mu\text{g}/0.1 \text{ ml}$) and 5 - fluorouracil ($130.0 \mu\text{g}/0.1 \text{ ml}$) applied during vernalization treatments.

Examining the data in Table III it is found that all plants headed within a period of 29 to 39 days with the exception of the unvernallized controls which headed in 140 days. Since the aim of this investigation was to attempt to offset, or delay vernalization, or to impede the progress



FIG 5. Photograph showing morphological effects produced by applications of chloramphenicol to vernalizing Sangaste fall rye seedlings.

1. Chloramphenicol (1.50 mg/0.1 ml) applied to vernalizing seedlings.
2. Chloramphenicol (1.50 mg/0.1 ml) applied to vernalized seedlings.
3. Unvernalized control plants.
4. Vernalized control plants.

to flowering, once vernalization had been completed, by antimetabolite treatment, an analysis of variance (Table 1V) was conducted for the data summarized in Table 111 using the unvernallized plants as controls. This revealed a significant difference at the 1% level between all treatments and the unvernallized control.

Taken all together the results show no marked effect on the vernalization process, or impotence of progress to flowering, by antimetabolite treatment.

With the exception of actinomycin D all the antimetabolites caused variation from normal growth. Fig 5 shows clearly the variable response of chloramphenicol treated plants. The control vernalized and control unvernallized plants are growing normally. The plants which received applications of 1.50 mg of chloramphenicol/0.1 ml following vernalization are somewhat shorter. On the other hand, plants which received applications of 1.50 mg of chloramphenicol/0.1 ml during vernalization have been seriously affected. Two of the plants have died, one of the surviving plants has 2 tillers and is beginning to head, the other has no tillers and is showing retarded growth. In addition, the upper leaves are of a distinctly longer and broader type than the control. They are also of a deeper green colour. The stalks are also affected being much thicker and greener than those of the controls.



FIG 6. Photograph showing morphological effects produced by applications of 5 - fluorouracil and 5 - fluorodeoxyuridine to vernalizing Sangaste fall rye seedlings.

1. 5 - fluorouracil ($130.0 \mu\text{g}/0.1 \text{ ml}$) applied to vernalizing seedlings.
2. 5 - fluorodeoxyuridine ($24.5 \mu\text{g}/0.1 \text{ ml}$) applied to vernalizing seedlings.
3. Unvernalized control plants.
4. Vernalized control plants.

Table V. Effect of protein and nucleic acid antimetabolites on number of tillers of Sangaste fall rye when applied during and after vernalization. (Tiller number during week of heading is underlined).

<u>Treatment</u>	<u>Weeks after planting</u>				
	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
Vernalized control	2.2	2.4	0.0	<u>0.0</u>	0.0
<u>a. Plants undergoing vernalization were treated 9 times with 0.1 ml containing</u>					
50% ethanol	2.2	2.2	0.2	<u>0.0</u>	0.0
water	2.0	2.3	0.5	<u>0.0</u>	0.0
<u>actinomycin D</u>					
50.00 μ g	2.4	2.4	0.3	<u>0.0</u>	0.0
12.50 μ g	2.5	2.5	0.2	<u>0.2</u>	0.0
6.25 μ g	1.8	2.6	0.7	<u>0.3</u>	0.0
<u>chloramphenicol</u>					
1.50 mg	0.9	1.4	1.9	<u>1.4</u>	0.7
1.00 mg	2.0	2.4	1.6	<u>0.9</u>	0.0
0.50 mg	2.8	3.1	1.0	<u>0.0</u>	0.0
<u>5 - fluorouracil</u>					
130.0 μ g	1.5	4.0	1.5	1.7	<u>0.5</u>
13.0 μ g	2.2	2.2	0.6	<u>0.0</u>	<u>0.0</u>
1.3 μ g	2.3	2.3	0.8	<u>0.2</u>	0.0
<u>5 - fluorodeoxyuridine</u>					
24.5 μ g	2.7	4.3	3.6	2.9	<u>1.3</u>
2.45 μ g	2.9	3.2	1.2	<u>0.9</u>	<u>0.1</u>
0.245 μ g	1.8	1.8	0.5	<u>0.2</u>	0.0
<u>b. Vernalized plants were treated 3 times with 0.1 ml containing</u>					
50% ethanol	2.3	2.6	1.0	<u>0.3</u>	0.0
water	1.7	2.2	0.9	<u>0.6</u>	0.0

Table V. (Con't)

<u>Treatment</u>	<u>Weeks after planting</u>				
	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>
<u>actinomycin D</u>					
50.00 μ g	1.8	2.1	0.3	<u>0.0</u>	0.0
12.50 μ g	2.2	2.3	0.7	<u>0.3</u>	0.0
6.25 μ g	2.6	2.7	0.3	<u>0.2</u>	0.0
<u>chloramphenicol</u>					
1.50 mg	1.2	1.6	1.2	<u>0.8</u>	0.0
1.00 mg	1.1	1.5	1.2	<u>0.4</u>	0.0
0.50 mg	1.8	2.2	1.2	<u>0.1</u>	0.0
<u>5 - fluorouracil</u>					
130.0 μ g	1.6	2.1	0.5	<u>0.4</u>	0.0
13.0 μ g	2.4	3.1	0.7	<u>0.0</u>	0.0
1.3 μ g	1.5	1.7	0.4	<u>0.0</u>	0.0
<u>5 - fluorodeoxyuridine</u>					
24.5 μ g	2.5	2.7	0.8	<u>0.1</u>	0.0
2.45 μ g	2.1	2.2	0.5	<u>0.0</u>	0.0
0.245 μ g	2.5	2.5	0.3	<u>0.0</u>	0.0

The data are an average of 3 replicates with 4 plants per replicate.

In this connection, Evans (1964) in his work with Lolium reported a growth retarding effect of chloramphenicol. Other effects of the antimetabolite were leaf damage, which was also observed by Collins et al. (1963) in their work with Cocklebur. Chlorophyll deficiency was also noted in sections of leaves and stalks during application of the antimetabolite. This may be explained by the work of Margulies (1962) who reported that chloramphenicol affects maturation of chloroplasts by affecting protein synthesis. This work has been further extended by Anderson and Smillie (1966) who have shown that chloramphenicol is more strongly bound by chloroplast ribosomes than by cytoplasmic ribosomes.

Fig 6 shows the variable response of 5 - fluorouracil and 5 - fluorodeoxyuridine treated plants. The control vernalized and control unvernallized plants are growing normally. The plants which received applications of 130.0 μ g of 5 - fluorouracil/0.1 ml and 24.5 μ g of 5 - fluorodeoxyuridine/0.1 ml during vernalization have been affected. Some plants have been killed, others were retarded in growth and headed when they were much shorter than the controls.

Table V shows that with few exceptions the period of maximum tillering was in the third week after planting. Following this, tiller number was in most cases rapidly reduced and in the week after heading few living tillers

remained. This may have been due to the fact that with a planting rate of 4 seedlings per 15 cm pot, and no addition of nutrients after planting, root development and availability of food material may have become limiting. As a consequence the parent plant would have been unable to support further tiller production as all available food material was diverted to development of the primary shoot.

Plants treated with the highest concentrations of 5 - fluorouracil and 5 - fluorodeoxyuridine while undergoing vernalization had the most tillers during the period of maximum tillering. In this connection Evans (1964) in his work with Lolium also reported increased tillering following application of these antimetabolites. It is of interest to note in Fig 6 that one of the 5 - fluorodeoxyuridine treated plants had 2 tillers heading at the same time as the main stalk.

In addition to the morphological effects described above, there was also the usual shortening of the lamina of the first leaf produced by vernalized cereals, observed by Thimann and Lane (1938), Čajlachjan and Zdanova (1938), Denffer (1939), Hänsel (1953), Purvis and Hatcher (1959) and others. This effect has been found to be a concomitant effect of low temperature and unrelated to vernalization.

In view of the morphological effects described



FIG 7. Photograph showing Sangaste fall rye plants which were treated with actinomycin D while undergoing vernalization.

1. Vernalized control plants.
2. Actinomycin D ($50 \mu\text{g}/0.1 \text{ ml}$) applied to vernalizing seedlings.
3. Unvernallized control plants.

above, it is not possible to say that the lack of effect of these antimetabolites on the number of days from planting to heading was due to insufficient uptake of antimetabolites. Neumann (1964) has reported that in lettuce seedlings shoot uptake of actinomycin D was extremely limited with consequently little effect on shoot processes, and this could also have been the case here.

In this study, plants treated with the highest concentration of actinomycin D headed 2 days earlier than the controls. The height differences shown in Fig 7 may be related to this age difference, as the photograph was taken after all the control plants had headed.

The results of this study imply that vegetative growth differs in some fundamental way from reproductive growth. Marked effects upon vegetative growth were observed with little or no effect upon time of flowering. Interference with the processes required for normal leaf and shoot growth may have little apparent effect on the growth and development of the floral bud.

The most severe morphological effects were exhibited by plants which had received applications of antimetabolites while undergoing vernalization. This can probably be explained by the fact that during this period the seedlings would be in an early stage of development, and hence, would be most sensitive to any

interference with their normal metabolism. By the end of vernalization their growth patterns would be well established and not so easily subject to alteration.

The results of this study are somewhat in disagreement with those of Dévay (1965) who has reported that chloramphenicol treatment of winter wheat resulted in a disturbed or incomplete vernalization. In her paper she first reported success in isolating an RNase which is synthesized within the first 24 hours of vernalization. On the basis of the temperature conditions and the effects of various inhibitors, including chloramphenicol, on its activity during vernalization, she stated that it most probably takes part directly in vernalization metabolism. Dévay continued her study by counting numbers of leaves produced by treated plants. She reported, that vernalization was disturbed or incomplete, based on the fact that the inhibitors used had a significant effect in producing plants with a higher leaf number than the vernalized controls. This effect was not noted in the present study as evidenced by a study of Figs 5, 6 and 7. Dévay did not report the number of days from planting to heading for the treatments used in her study, so it is not possible to make any comparison on this basis with the results of this investigation. However, if Dévay's findings are in fact related to the vernalization response, then lack of effect of inhibitors applied in this study on the vernalization reaction, could be attributed to their late application. Dévay's method of

application was to soak ungerminated seeds in inhibitor and then spray the resulting seedlings twice during vernalization. In the present study antimetabolite application did not commence until 11 days after germination. This would mean that Dévay's essential RNase had already been synthesized. In such a case only suppression of nucleic acid replication would have resulted until antimetabolite treatment had been terminated. Following this normal replication of nucleic acids and protein synthesis would resume leading to early heading as was observed. Such reasoning, however, would not explain why a period of several weeks of low temperature treatment is necessary for complete vernalization to be effected. Dévay's results explain this by indicating a second maximum of RNase activity at a later stage in vernalization which is less affected by inhibitors. She attributes this to realignment or activation of this enzyme.

Section IV. Changes in Lipids During Vernalization

Materials and Methods

Seven 50 gram lots of each of Sangaste fall rye, Prolific spring rye, Kharkov winter wheat and Red Bobs spring wheat were disinfected with 0.3% hydrogen peroxide solution for 5 minutes. They were then washed several times with sterile distilled water and soaked in sterile distilled water for 2 hours. Following this, the seeds were placed in sterile plastic dishes containing blotting paper moistened with 25 ml of sterile distilled water. These dishes were covered with aluminum foil in which 4 holes were punched to permit aeration, and placed in a dark room maintained at 4 to 6°. The moisture content of the dishes was kept at a constant level by regular weighings and additions of sterile distilled water. The materials were collected at 0 (dormant seed), 1, 2, 3, 4, 5, and 6 weeks.

At times of harvesting several seedlings of each variety were selected at random and grown to maturity in a growth chamber as described in section I. Dormant seeds planted at 0 weeks served as controls. After removal of any dead or diseased material, the remainder was dried in a vacuum oven at 60° for 2 days. The dried material was ground in a Wiley mill to pass a 20 mesh sieve and stored in brown screw cap bottles in a deep freezer at -20° until required.

The entire experiment was repeated twice. Each time 2 batches of material were grown and combined for analysis.

a. Extraction of Lipids

The method used was a modification of that described by Mihailović et al. (1963).

Ten to 12 gram of sample from each lot of material was dried to constant weight in a vacuum oven at 60°. To each sample was added 150 ml of 2 : 1 chloroform - methanol mixture. The mixture was well shaken and allowed to stand overnight at room temperature. After refluxing gently for 3 to 4 minutes it was filtered through a 10 to 20 μ sintered glass funnel. The filtrate was distilled almost to dryness on a water bath, the residual solvent being removed in a nitrogen stream. The residue was taken up in ethanol - free chloroform (prepared by refluxing reagent grade chloroform with calcium chloride for 45 minutes and distilling the mixture) and filtered through a layer of Hyflo Super-Cel on a 10 to 20 μ sintered glass funnel. This procedure removed the bulk of the protein and starch impurities. The bulk of the solvent was removed by distillation on a water bath and the remainder in a nitrogen stream. Further purification was effected as follows. The residue was treated with hot methanol which was removed in a nitrogen stream. It was then dissolved in ethanol - free chloroform and filtered through a layer of anhydrous sodium sulfate on

a 10 to 20 μ sintered glass funnel. This filtration removed solid particles as well as water traces. The ethanol - free chloroform was then removed in a nitrogen stream and the residue weighed. This treatment was continued until constant weight was obtained and this was taken as the weight of total lipids.

Total lipid extracts were dissolved in ethanol - free chloroform and stored under nitrogen in a deep freezer at -20⁰ until required.

b. Fractionation of Lipids

Separation of lipids into non-polar and polar lipid fractions was effected on silicic acid columns. Silicic acid columns were 2.5 cm in diameter and 15 cm in height. The lower end of each column was fitted with a 25 to 50 μ sintered glass funnel and stopcock. The filter was cleaned periodically by passage through it of a hot concentrated sodium hydroxide solution.

Two hundred gram lots of silicic acid (100 mesh, for chromatography of lipids from Mallinckrodt, New York) were prepared each time column renewal was effected. The silicic acid was placed in a 1000 ml graduated cylinder. With thorough mixing methanol was added to the 1000 ml mark and the mixture allowed to stand for 30 minutes. At the end of this time the methanol, together with any particles of silicic acid which had not settled out, were decanted. This process was repeated twice. The silicic acid was then

washed thoroughly with ethanol - free chloroform and acetone and dried in an oven at 105⁰ for 2 hours. Batches of Hyflo Super-Cel were also washed and dried in the same way.

To each column was added a mixture of 20 grams of silicic acid and 10 grams of Hyflo Super-Cel suspended in ethanol - free chloroform. The mixture in the columns was allowed to settle overnight. The ethanol - free chloroform was drained to the level of the surface of the silicic acid - Hyflo Super-Cel mixture. The total lipid extract was now applied. The non-polar lipids were eluted with 200 ml of ethanol - free chloroform and the polar lipids with 200 ml of a 1 : 2 chloroform - methanol mixture.

The non-polar lipid fraction was distilled almost to dryness on a water bath and the residual solvent was removed in a nitrogen stream. After weighing this fraction was taken up in ethanol - free chloroform and stored under nitrogen in a deep freezer at -20⁰ until required.

Because silicic acid is slightly soluble in methanol, the residue remaining after removal under nitrogen of the solvent from the polar lipid fraction was further purified. This was effected by dissolving it in ethanol - free chloroform and filtering it through a layer of anhydrous sodium sulfate on a 10 to 20 μ sintered glass funnel. The filtrate was distilled almost to dryness on a water bath and the residual solvent was removed in a nitrogen stream. The polar lipids

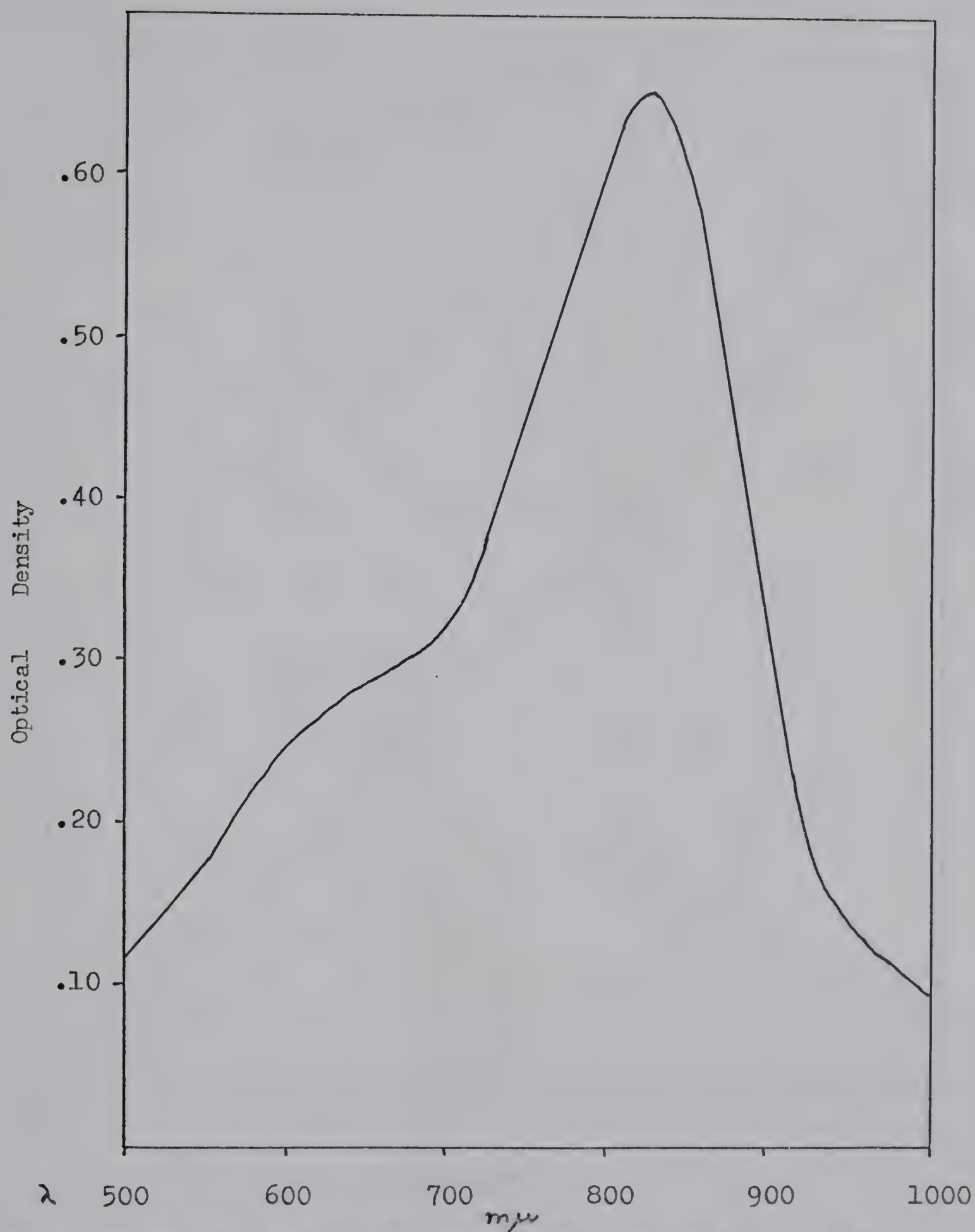


FIG 8. Spectrum of the phosphomolybdate complex produced during determination of phosphorus by the method of Bartlett (1958).

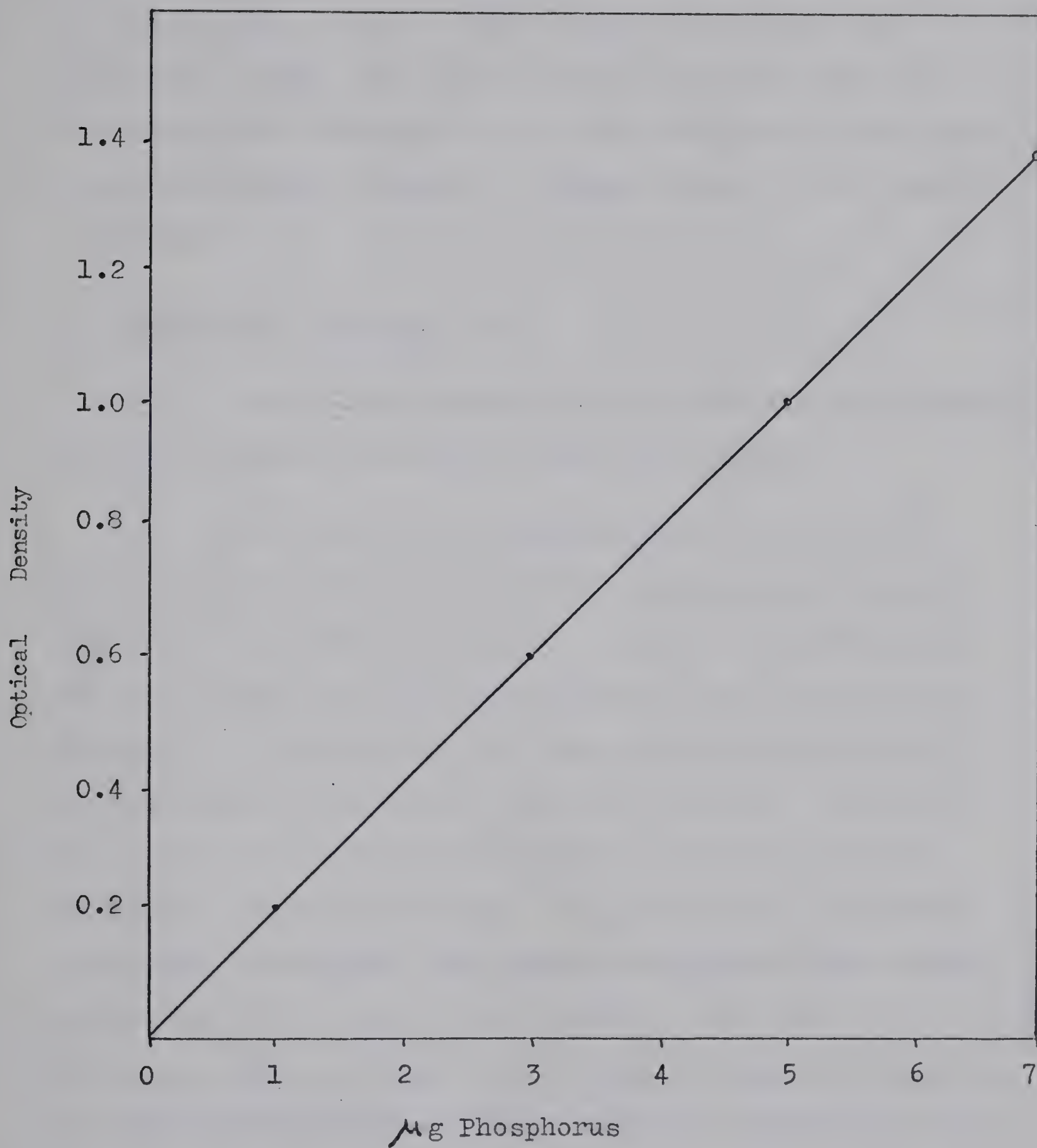


FIG 9. Standard curve for phosphorus determinations. The standards were prepared by the method of Bartlett (1958).

were then determined gravimetrically. They were made up to volume with ethanol - free chloroform in a 10 ml volumetric flask, from which 0.05 ml portions were taken for phosphorus determinations. The remainder of the solution was stored under nitrogen in a deep freezer at -20° until required.

c. Phosphorus Determinations

Polar lipid phosphorus determinations were carried out by the method described by Bartlett (1958).

The sample to be analyzed and 0.5 ml of 10 N sulfuric acid were placed in a 20 ml pyrex test tube and heated in a 150 to 160° oven for 3 hours. Several drops of 30% hydrogen peroxide were added and the solution was returned to the oven for 1.5 hours more to complete the combustion and to decompose all the peroxide. Four point six ml of 0.22% ammonium molybdate and 0.2 ml of Fiske - Subba Row reagent were added. The contents of the tubes were mixed thoroughly, the tubes were covered with marbles and heated for 7 minutes in a boiling water bath. The optical density at $830\text{ m}\mu$ (which is the region of maximum absorbance of the phosphomolybdate complex produced, as shown in Fig 8) was recorded with a Beckman DK-1 spectrophotometer. The concentration of phosphorus in the sample was then determined from the standard curve (Fig 9).

d. Gas Liquid Chromatography

Non-polar and polar lipid fractions were prepared for gas liquid chromatography as follows.

The ethanol - free chloroform was removed from the samples in a nitrogen stream and they were saponified for 16 hours with 5 ml 1 N methanolic potassium hydroxide at 50°. One volume of water was added and the unsaponifiable material was extracted with several small portions of diethyl ether. Attempts were made to determine gravimetrically the amount of unsaponifiable material. These attempts were however not successful. This was especially so in the case of the polar lipids where the boundary between the diethyl ether and the aqueous phase was obscured by what could have been residual protein. Due to the extremely small amounts of unsaponifiable material unavoidable errors in extraction or inclusion of variable amounts of the boundary residue led to errors of large magnitude when calculations as % of lipid fraction was made.

The solution remaining, after extraction of the unsaponifiable material, was acidified with 1 ml of 10 N sulfuric acid. The liberated fatty acids were then extracted with several small portions of diethyl ether. The ether extracts were washed twice with water, filtered through anhydrous sodium sulfate to remove water traces and then placed in screw cap vials for esterification. The ether was removed under nitrogen, 3 ml of boron trifluoride (140 gram boron trifluoride/l of methanol)

were added and the mixture heated under nitrogen for 2 minutes in a boiling water bath. The mixture was diluted with 2 volumes of water, the fatty acid methyl esters were extracted with pentane, and after filtration through anhydrous sodium sulfate to remove water traces, were stored under nitrogen in a deep freezer at -20° until required.

The fatty acid methyl esters were analyzed on an Aerograph Model 600 gas chromatograph equipped with a hydrogen flame ionization detector. A coiled copper column 10'x 1/8" packed with diethylene glycol succinate on 60 to 80 mesh chromosorb P was employed. The operating conditions were: column temperature 200° , injector temperature 250° and flow rate of nitrogen carrying gas 25 ml per minute. At least 2 analyses were made for each sample. The peaks were identified by matching known fatty acids with unknowns.

e. Thin Layer Chromatography

The absorbent used was silica gel G according to Stahl for thin layer chromatography (Merck, G. A., Darmstadt, Germany). Thirty grams of silicic acid were mixed thoroughly with 60 ml of distilled water. This slurry was sufficient to coat nine 10 x 20 cm plates. The plates were air dried for 1 hour and activated for 1 hour just before use.

Lipids were applied to the plates with a 10 μ l syringe. One dimensional ascending development of the spots was then carried out. Chloroform was used in the case of the non-polar lipids and a 65 : 25 : 4 chloroform - methanol - water mixture in the case of the polar lipids. The lipid

spots were made visible by spraying with 50% sulfuric acid and heating the plates at 80° for 15 minutes. The plates were compared to determine if there was any visible difference between lipids of varieties or treatments.

Results and Discussion

a. Materials and Methods

Both rye and wheat were used in this study so as to be able to determine if lipid trends varied markedly between the two. Winter and spring varieties of each were used so that any observable trends might not be mistakenly attributed as being related to the vernalization response.

Attempts were made before and during growth of the cereals in low temperature to keep conditions as sterile as possible. These efforts were essentially successful.

Vernalization was effected in complete darkness to ensure that there was no formation of chlorophyll. Chlorophyll is soluble in the organic solvents used for lipid extraction and would have introduced a further variable into the results, since, under the experimental conditions used for vernalization regulation of light intensity would have been extremely difficult.

Since a relatively high temperature was used for vernalization, moisture content was restricted to slow the growth of the cereals and thus avoid exhaustion of endosperm food material.

Cereal grain lipids are very complex products (Garton et al. 1963) and their intensive and detailed study has only recently begun. The difficulties encountered in their isolation and separation together with the fact that they occur in small quantity has made their study an area to be avoided by most researchers.

Investigations by Sullivan (1940) and Zentner (1958) have shown that more than half of the lipids in wheat flour are firmly bound to the proteins in the form of lipoprotein complexes. Rohrllich and Niederauer (1967) have isolated similar lipoprotein complexes from rye.

Various methods have been suggested for the extractions of total lipids and the disadvantages of solvents such as n-butanol, petroleum ether, ethyl ether or ethanol are well known (Tsen et al. 1962). Of these solvents, water saturated n-butanol is the most satisfactory, especially since it readily decomposes the lipoprotein complexes (Mecham and Mohammad, 1955). But because of its high boiling point, this solvent may cause oxidation of unsaturated acids, and is, therefore, not convenient when further fatty acid analysis is necessary.

In this investigation the chloroform-methanol solvent mixture in the proportions originally proposed by Folch et al. (1957) was used. However, since a high amount of starch and protein was extracted together with the lipids, it was necessary to evaporate the extract to

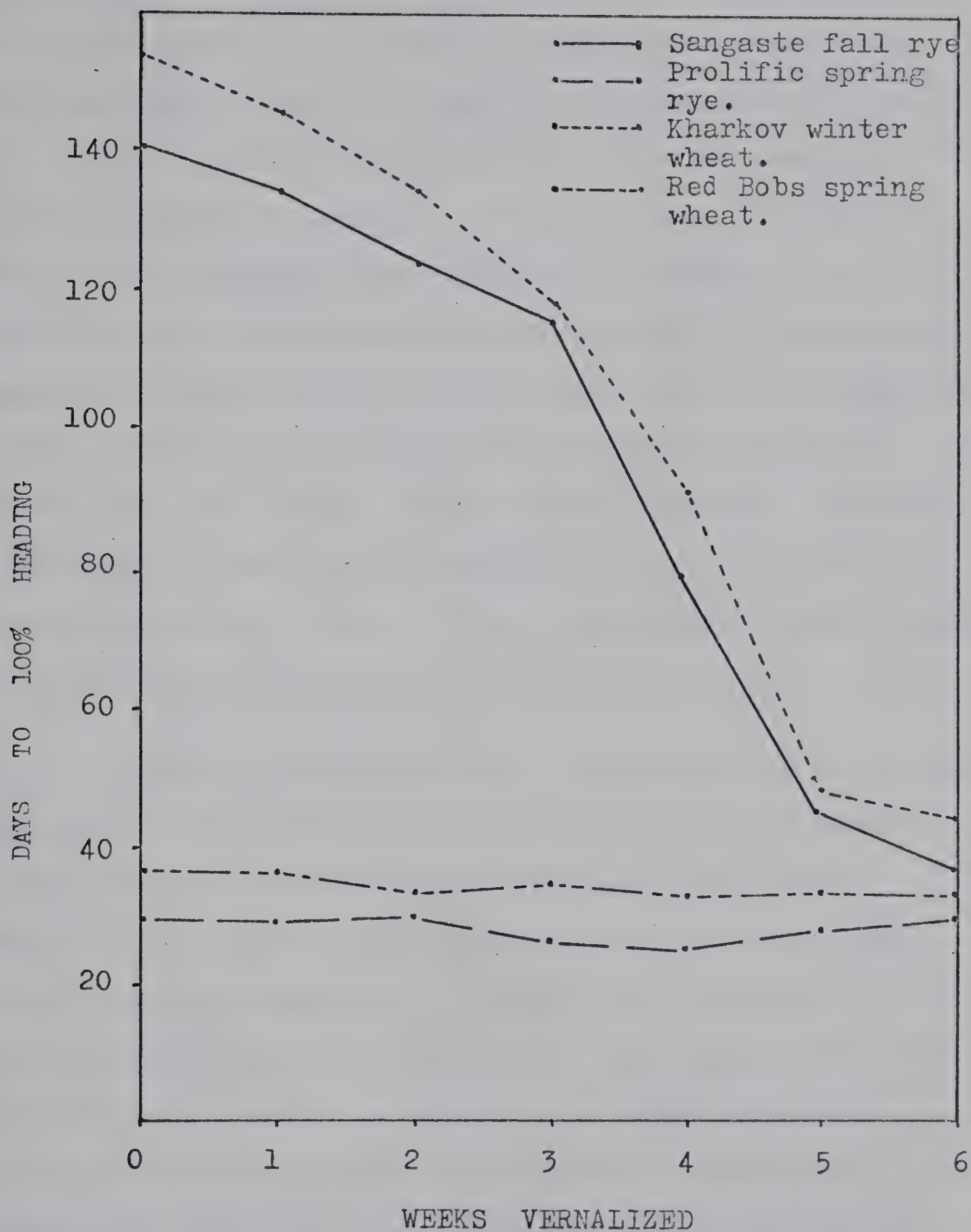


FIG 10. Relationship of number of days to 100% heading to duration of vernalization of cereal varieties.

Each point on the graph represents the average of 3 replicates with 4 plants per replicate.

dryness and to re-extract the residue with ethanol-free chloroform. The lipids isolated under these conditions still contained a variable amount of protein impurities and it was, therefore, necessary to treat the residue vigorously with hot methanol. Nevertheless, the residue obtained from methanol and dissolved in ethanol-free chloroform still contained a certain amount of lipoprotein complex. However, since in each case methanol treatment was applied until constant weight was attained, it may be assumed that this amount was no longer variable. Washing of the lipid extract by the method of Folch et al. (1957) was not carried out since it has been shown by Rouser et al. (1963) and Mihailovic et al. (1963) that lipid loss occurs.

During fractionation of total lipids, it was found that unless fine particles of silicic acid were removed by the sedimentation procedure previously described, packing of the column and plugging of the sintered glass filter occurred resulting in lengthy elution times. It was also found advisable to prepare a new column each time a fractionation ended. Apparently, after methanol washes to clean the column a monolayer of alcohol was tenaciously retained (Wren, 1960) and can cause variable results.

b. Vernalization

The vernalization curves in Fig 10 show that spring rye and spring wheat did not respond to low

Table VI. Changes in total lipids and lipid fractions of cereal varieties during vernalization.

<u>Variety</u>	<u>Weeks of vernalization</u>	<u>Total lipids as % of dry sample</u>	<u>Polar lipids as % of total lipids</u>	<u>Non-polar lipids as % of total lipids</u>
Sangaste 0(dormant fall rye. seed)		1.8	29.4	70.6
	1	1.8	29.0	71.0
	2	1.8	32.1	67.9
	3	1.7	35.8	64.2
	4	1.8	43.8	56.2
	5	2.0	45.3	54.7
	6	2.1	42.6	57.4
Prolific 0(dormant spring rye. seed)		1.9	28.8	71.2
	1	1.8	28.5	71.5
	2	1.8	31.9	68.1
	3	1.7	36.6	63.4
	4	1.7	41.9	58.1
	5	1.9	42.0	58.0
	6	2.1	45.2	54.8
Kharkov 0(dormant winter wheat. seed)		2.5	29.3	70.7
	1	2.3	30.2	69.8
	2	2.1	30.5	69.5
	3	2.1	35.9	64.1
	4	1.9	46.3	53.7
	5	1.8	48.3	51.7
	6	1.9	45.9	54.1
Red Bobs 0(dormant spring wheat. seed)		2.5	27.2	72.8
	1	2.4	25.3	74.7
	2	2.1	30.6	69.4
	3	2.0	36.4	63.6
	4	2.0	46.7	53.3
	5	1.9	44.9	55.1
	6	2.1	46.3	53.7

The data are an average of 6 separate determinations. Three determinations on each of 2 samples.

temperature treatment as regards reductions of number of days from planting to heading. The spring rye, however, consistently headed earlier than the spring wheat. The fall rye and winter wheat, on the other hand, showed a marked response to the low temperature treatment. Until the third week of treatment response was rather slow. After this there was a rapid decrease in the number of days from planting to heading with the fall rye heading consistently earlier than the winter wheat.

c. Total Lipids

It should be emphasized that the data represents the net changes for entire seedlings. It is quite possible and in fact probable that lipids were catabolized in one tissue of the seedling and resynthesized in another tissue simultaneously.

As may be observed in Table VI all the cereal varieties examined had relatively low lipid contents. It would be suspected, therefore, that lipids would not play any major role as food material during germination, and this appeared to be the case. During germination there was a slight decrease in total lipids followed by a slight increase. In the wheat varieties the increase occurred after the fifth week of low temperature treatment. A similar trend was reported by David (1951) in his studies on growth of wheat at low temperature. In the rye varieties the increase of total lipids occurred somewhat earlier, in fall rye after the third week and in the spring rye after the fourth week of low temperature

treatment. These differences are probably a reflection of the differences in growth rate and/or metabolic rate between the varieties under low temperature conditions. The ryes have been observed to grow at a much faster rate than the wheats and, therefore, any observable changes in composition would be expected to occur earlier in the faster growing varieties.

Fractionation of the total lipids showed that there was little to distinguish between the varieties. During treatment there was an overall increase in the polar lipid fraction coupled with a corresponding decrease in the non-polar lipid fraction. It should be noted that this inverse relationship exists as a consequence of the method of analysis. However, in view of the fact that the plants were growing, and that the % changes of total lipids are much smaller than the % changes of the fractions, it may be assumed that the changes represent synthesis of polar lipids for incorporation into plant structures, from non-polar lipids. Pomerantz et al. (1966) have reported that these are composed mainly of triglycerides. Such synthesis would probably have occurred according to the scheme given by Mihailović et al. (1963).

The increase of polar lipids and decrease of non-polar lipids were not gradual during the period of growth, but occurred as a series of rapid increases over a short period of time. This was especially marked in the wheat varieties, where between the third and fourth weeks of

Table VII. Changes in polar lipid phosphorus of cereal varieties during vernalization.

<u>Variety</u>	<u>Weeks of vernalization</u>	<u>Polar lipid phosphorus as mg/100 grams of sample</u>	<u>Phospholipids as % of polar lipids</u>
Sangaste 0(dormant fall rye. seed)		6.5	30.1
	1	7.1	30.2
	2	8.3	30.2
	3	9.1	30.7
	4	10.2	30.1
	5	10.1	31.3
	6	10.4	31.4
Prolific 0(dormant spring rye. seed)		6.4	29.6
	1	7.0	31.0
	2	8.4	30.4
	3	8.5	30.1
	4	9.8	31.1
	5	10.1	31.6
	6	10.3	30.3
Kharkov 0(dormant winter wheat. seed)		7.1	27.9
	1	7.4	27.4
	2	7.5	30.1
	3	8.4	30.6
	4	10.2	30.7
	5	10.2	30.8
	6	10.4	30.0
Red Bobs 0(dormant spring wheat. seed)		7.1	28.4
	1	7.2	27.8
	2	8.2	31.6
	3	8.2	30.2
	4	9.6	30.9
	5	10.9	30.8
	6	10.8	31.3

The data are an average of 4 separate determinations each determination being done in duplicate.

treatment the polar lipids increased by approximately 10% out of an overall 19% increase, while the non polar lipids decreased by a corresponding amount. These periods of sudden change must have represented periods of rapid metabolism in the seedlings when the production of structural material was at a maximum.

d. Phosphorus Determinations

In Table VII phospholipids as a % of polar lipids were calculated by the method of Kates and Baxter (1966). Assuming an average molecular weight for phospholipids of 800, the % phospholipids = % phosphorus of polar lipids x 25.

The polar lipid phosphorus expressed as mg/100 grams of dry sample showed an overall increase during treatment, whereas the phospholipids expressed as % of polar lipids showed very little change. These results indicate that the phospholipids increased progressively with an increase of the polar lipid fraction, since, the phospholipids were always a relatively constant % of the polar lipid fraction which showed a general increase throughout the low temperature treatment (Table VI). It is not possible to say on the basis of this investigation, whether the increase was due to an increase in only one phospholipid or whether all phospholipids increased concurrently. Here again the results for all the varieties were similar. It is of interest to note that Weiss (1952) has reported that phospholipid phosphorus showed very little change in

Table VIII. Changes in the major fatty acids of the polar lipid fractions of cereal varieties during vernalization. (The data are in % of the total peak area of the major fatty acids)

Variety	Weeks of vernalization	Major fatty acids				
		16:0	18:1	18:2	18:3	18:1 + 18:2
Sangaste fall rye.	0(dormant seed)	20.8	15.3	58.5	5.4	73.8
	1	20.6	14.2	55.0	10.2	69.2
	2	20.5	11.5	51.7	16.3	63.2
	3	21.5	10.2	49.6	18.7	59.8
	4	22.2	9.8	41.8	26.2	51.6
	5	22.7	7.7	41.9	27.7	49.6
	6	20.7	12.2	40.2	26.9	52.4
Prolific spring rye.	0(dormant seed)	22.4	15.2	56.9	5.5	72.1
	1	21.4	13.5	50.3	14.8	63.8
	2	21.4	12.3	51.2	15.1	63.5
	3	22.4	10.2	50.6	16.8	60.8
	4	21.3	9.5	42.3	26.9	51.8
	5	21.2	9.4	42.9	26.5	52.3
	6	21.4	8.8	39.4	30.4	48.2
Kharkov winter wheat.	0(dormant seed)	18.0	13.7	63.7	4.6	77.4
	1	21.4	9.9	61.1	7.6	71.0
	2	21.1	8.8	59.1	11.0	67.9
	3	20.6	10.0	58.6	10.8	68.6
	4	20.5	7.8	55.7	16.0	63.5
	5	20.1	9.9	56.0	14.0	65.9
	6	20.0	8.1	60.2	11.7	68.3
Red Bobs spring wheat.	0(dormant seed)	19.2	16.3	61.0	3.5	77.3
	1	21.5	12.7	58.3	7.5	71.1
	2	21.5	12.6	56.4	9.5	69.0
	3	21.9	10.9	57.5	10.7	68.4
	4	21.9	7.5	52.9	17.7	60.4
	5	22.4	9.3	54.6	13.7	63.9
	6	22.9	7.8	55.4	13.9	63.2

The data represents the averages of 4 separate determinations. Two analyses on each of 2 samples.

Table 1X. Changes in the major fatty acids of the non-polar lipid fractions of cereal varieties during vernalization.

Variety	Weeks of vernalization	Major fatty acids				
		16:0	18:1	18:2	18:3	18:1 + 18:2
Sangaste fall rye.	0(dormant seed)	18.2	24.5	49.5	7.8	74.0
	1	16.2	22.3	50.5	11.0	72.8
	2	16.1	20.6	50.1	13.2	70.7
	3	17.4	17.7	51.1	13.8	68.8
	4	18.3	14.9	48.4	18.4	63.3
	5	21.3	12.7	44.8	21.2	57.5
	6	21.1	11.6	40.9	26.4	52.5
Prolific spring rye.	0(dormant seed)	20.1	24.2	48.2	7.5	72.4
	1	19.0	22.5	47.9	10.6	70.4
	2	18.0	21.3	49.3	11.4	70.6
	3	17.9	19.2	49.2	13.7	68.4
	4	19.1	15.8	47.2	17.9	63.0
	5	19.3	15.7	47.0	18.0	62.7
	6	19.4	14.2	43.6	22.8	57.8
Kharkov winter wheat.	0(dormant seed)	21.1	22.5	52.9	3.5	75.4
	1	20.5	22.2	53.1	4.2	75.3
	2	18.7	21.4	53.6	6.3	75.0
	3	19.4	19.3	53.7	7.6	73.0
	4	21.3	16.5	50.7	11.5	67.2
	5	21.3	14.4	50.3	14.0	64.7
	6	21.8	13.0	50.9	14.3	63.9
Red Bobs spring wheat.	0(dormant seed)	20.7	22.9	53.7	2.7	76.6
	1	20.4	23.7	52.5	3.4	76.2
	2	20.0	21.3	54.5	4.2	75.8
	3	21.1	18.2	54.8	5.9	73.0
	4	19.2	15.5	53.4	11.9	68.9
	5	19.1	16.1	53.3	11.5	69.4
	6	18.6	15.6	53.9	11.9	69.5

The data represents the averages of 4 separate determinations. Two analyses on each of 2 samples.

wheat and rye during 4 to 12 days germination at normal growing temperatures.

e. Gas Liquid Chromatography

Gas liquid chromatography of the polar and non-polar lipid fractions revealed the presence of major amounts of: Palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), and minor amounts (less than 1% of total peak area) of caproic acid (6:0), caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), pentadecanoic acid (15:0), palmitoleic acid (16:1), heptadecanoic acid (17:0) and stearic acid (18:0) in all varieties throughout growth.

The results given in Tables VIII and IX are expressed as fatty acids in % of total peak area of the major components being reported.

It may be seen that there was a considerable change with time in the amount of 18:3 acid. This acid showed an overall increase in all varieties in both the polar and non-polar fractions. The increase was especially marked in the rye varieties as compared with the wheat varieties, being most noticeable in the case of polar lipid fractions where the overall increase was 2 to 3 times as great in the rye varieties as in the wheat varieties. It is of interest to note that during the first week of growth there is, in the polar lipid fractions, a sudden

large increase in the proportion of 18:3 acid. This increase again occurs during the third and fourth weeks of growth. In the case of the non-polar lipid fractions only the third to fourth week increase is really marked.

The predominance of 18:3 acid during growth of the rye varieties as compared with the wheat varieties, could very well be a reflection of the cold hardiness of rye as compared with wheat. Lyons et al., (1964) obtained functional mitochondria from several plant species which differed in sensitivity to chilling injury. They then studied the physical properties of the mitochondrial membranes with light scattering techniques and they conducted fatty acid analysis. They found that the mitochondria from chilling-resistant tissues were more flexible and contained a higher amount of unsaturated fatty acids than did mitochondria from chilling-sensitive plant species. The degree of unsaturation showed a substantial range of values in accordance with mitochondrial swelling. The authors suggested that metabolic injury caused in chilling-sensitive tissues may have been due to the inability of relatively inflexible mitochondria to function at low temperatures. Further evidence of the role of fatty acids in cold hardiness was provided by Gerloff et al., (1966). They showed that the fatty acid content of alfalfa roots increased approximately two-fold during hardening due to the preferential accumulation of polyunsaturated fatty acids.

As regards the other major fatty acids there was an overall decrease in 18:1 acid, however, this overall decrease was not very marked between varieties. The 18:2 acid of the non-polar lipid fractions did not show an extreme range of values, but was always slightly higher in the wheat varieties than in the rye varieties. In the case of the polar lipid fraction, the 18:2 acid content of the wheat varieties was again somewhat higher than that of the rye varieties, but here there was a much greater decrease in the 18:2 acid content in the rye varieties than in the wheat varieties during treatment. There was a very little change in the 16:0 acid in any of the varieties either in the polar or non-polar lipid fractions.

The results of the present study further show a progressive increase of unsaturation of lipids with growth at low temperature. The influence of environmental temperature on the degree of unsaturation of lipids has been the subject of much investigation and discussion. Leathes and Raper, 1925; Gaughran, 1947; Deuel, 1955; Huber and Zalik, 1963; and Hilditch, 1964 have treated this aspect fully.

Although the data presented are of a semiquantitative nature the general picture is that there is an inter-conversion between 18:1, 18:2 and 18:3 acids. The trends are evident, with an increase of 18:1 + 18:2 there is a decrease of 18:3 and vice versa. Due to the method

of presentation of the data, and the fact that the 16:0 acid remains relatively constant such an inverse relationship might be expected to be observed. However, that such an interconversion can very well occur has been demonstrated by the radioisotopic studies of Huber and Zalik (1963), Stumpf and James (1963), McMahon and Stumpf (1964), and Harris and James (1965) and others.

Besides the major acids which have just been discussed there was also noted the presence of minor acids. The appearance of these minor acids in the various fractions was probably due to the fact that lipids were in a dynamic state and were continually being hydrolyzed to glycerol and free fatty acids and resynthesized. An alternate explanation was that the catabolism and anabolism of the lipids was occurring simultaneously in two separate tissues within the seedling, for example catabolism in the endosperm and anabolism in the shoot. Since the different tissues were not separated prior to analysis, it cannot be stated definitely which of the above explanations is correct.

The occurrence of odd number carbon acids 15:0 and 17:0 which have also been reported in wheat flour by Garton et al. (1963) indicates the presence of the α -oxidation system which at the present time is the only known mechanism to account for the presence of odd number carbon fatty acids in higher plants.

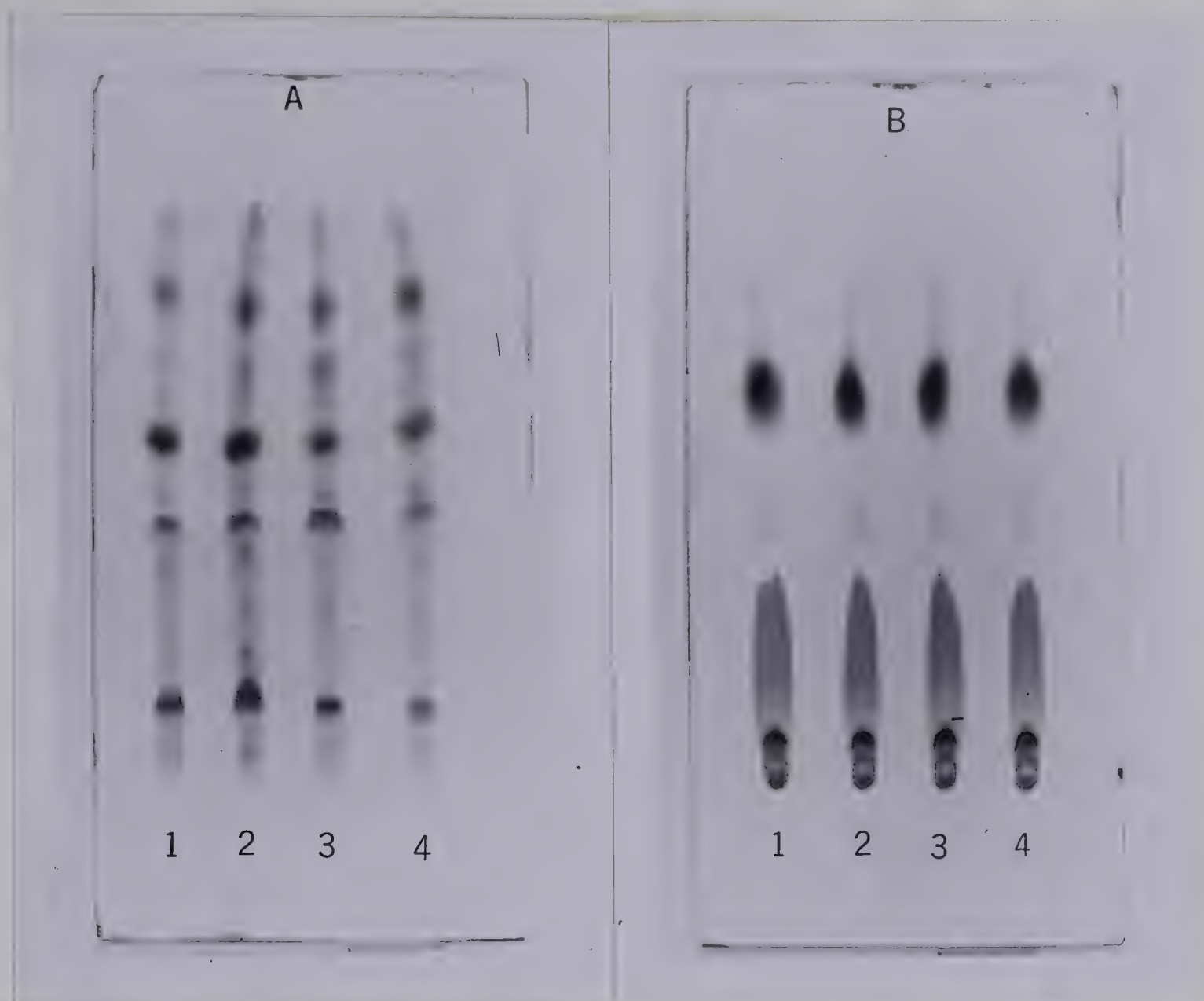


FIG 11. Photographs showing examples of the type of results obtained at each stage of vernalization by thin layer chromatography of polar and non-polar lipid fractions.

- A. Polar lipid fraction.
- B. Non polar lipid fraction.
- 1. Sangaste fall rye.
- 2. Prolific spring rye.
- 3. Kharkov winter wheat.
- 4. Red Bobs spring wheat.

f. Thin Layer Chromatography

Thin layer chromatography revealed no qualitative differences between either varieties or weeks of low temperature growth. Figs 11A and B are examples of typical result obtained at each stage of vernalization.

It is of interest to note that throughout this investigation marked changes in lipids invariably occurred during the 3 to 4 week stage of vernalization. This was the stage of vernalization where the vernalizing fall rye and winter wheat showed a marked response to low temperature as regards reduction in number of days to heading. However, in view of the fact that such changes in lipids also occurred in spring varieties, which show no flowering response to low temperature, it has to be concluded that these changes are simply a response to low temperature conditions and bear no direct relationship to the vernalization process.

GENERAL DISCUSSION AND CONCLUSIONS

In order to conduct an investigation on vernalization it is first necessary to test the method to be used. This is essential, because as has already been discussed in the literature review, successful vernalization is dependent on many factors. The method of vernalization used was shown to be effective both under conditions of light and darkness. In the case of dark vernalization, however, moisture content of the grain was regulated to 50% of the dry weight to ensure that too rapid growth and subsequent exhaustion of endosperm food material did not occur. This was successful, and there was continued response to low temperature during the vernalization period.

Attempts to induce earlier heading in unvernallized Sangaste fall rye by application of vernalized fall rye seedling extract and nucleic acids were not successful. Some reasons were suggested for failure. The solvents used may not have extracted the so called active vernalization product or may have inactivated it. The substance may not have been absorbed by the test plants, or at least not in sufficient quantity to produce an effect. Another possibility is that there is in fact no active substance.

Treatment of vernalizing and vernalized fall rye seedlings with protein and nucleic acid antimetabolites did not have any marked effect on the vernalization process, or impedance of progress to flowering. Duncan's Multiple Range

Test (1955) revealed that although significant differences did exist between various treatments no overall pattern could be distinguished. The only treatment that differed significantly from the vernalized control was the 5 - fluorouracil ($130 \mu\text{g}/0.1 \text{ ml}$) treatment applied to plants undergoing vernalization. However, there were marked morphological effects. These results suggested that there is some fundamental difference between the nature of vegetative growth and that of reproductive growth. On the other hand, Dévay (1965) reported that there was an increase in final leaf number of plants which were treated with protein and nucleic acid antimetabolites while undergoing vernalization. She interpreted this as a disturbance or interruption of vernalization, and related it to an inhibition of a specific RNase synthesized early in the vernalization process. Such an effect on final leaf number was not observed in the present study.

If Dévay's interpretations are accepted, then the lack of effect of antimetabolites on reproductive growth in the present study, could be attributed to their not being applied early enough to affect the synthesis of the specific RNase.

During low temperature growth of fall rye, spring rye, winter wheat and spring wheat, changes in lipids were observed. In most instances, however, changes were similar between varieties and could not be used to distinguish

between them.

Total lipids showed an initial decrease with an increase toward the end of the period of low temperature growth. Polar lipids showed an overall increase and there was a corresponding decrease in non polar lipids. This suggested a conversion of non-polar lipids into polar lipid structural material.

Polar lipid phosphorus expressed as mg/100 grams of dry sample showed an overall increase, while the phospholipids expressed as % of polar lipids showed little change. This indicated that there was a progressive increase of phospholipids with the increase of the polar lipid fraction.

Gas liquid chromatography revealed the presence of many major and minor fatty acids including the odd number carbon acids 15:0 and 17:0. The presence of these odd number carbon acids indicated the operation of the α -oxidation system. Marked differences were observed between the rye and wheat varieties in the proportions of 18:2 and 18:3 acids. The 18:3 acid was always higher in rye than in wheat, whereas the reverse was true for the 18:2 acid. This difference was suggested as being related to the greater cold hardness of rye.

The well documented increase of unsaturation of fatty acids with growth at low temperature was noted. It was suggested that in view of the trends observed there was

probably an interconversion of 18:1, 18:2 and 18:3 acids occurring along the lines indicated by the findings of Huber and Zalik (1963), McMahon and Stumpf (1964), Harris and James (1965) and others.

Thin layer chromatography of polar and non-polar lipid fractions revealed no qualitative differences between either varieties or weeks of low temperature growth.

Throughout the lipid analyses there was consistently a marked change occurring at the 3 to 4 week stage of low temperature growth. When these results were compared with the vernalization response curves it was evident that this was the time at which the winter varieties showed a marked response to the low temperature. Had winter varieties alone been analyzed it is probable that the observed changes would have been assumed to be related to the vernalization response. However, with spring varieties which do not respond to the low temperature stimulus included as controls, such a conclusion could not be arrived at as these also showed a similar trend. It must therefore be concluded that the observed changes were not related to the vernalization response but were rather a response to low temperature growth.

On the basis of the findings of this study, taken together with the findings of numerous reports in the literature, it is concluded that the vernalization mechanism is probably an extremely complex one. From a review of the literature it may be deduced that carbohydrates, proteins,

auxins, gibberellins, nucleic acids and specific substances, the production of which may be induced by low temperature, are all possibly involved in vernalization. It might well be that vernalization is the result, not of a change in any one of these factors, but of a simultaneous change in several factors. Vernalization would then result when a certain balance or relationship had been established between these factors. This relationship might vary for different plants so that each variety would need to be considered separately.

LITERATURE CITED

- ANDERSON, L. A., and R. M. SMILLIE. 1966. Binding of chloramphenicol by ribosomes from chloroplasts. Biochem. Biophys. Res. Comm. 23:535-539.
- AKSENOVA, O. F. 1960. Details of the change in winter wheat during vernalization and wintering. Uchenye Zapiski Tomsk. Gosudarst Univ. im. V. V. Kuibysheva No. 36:219-228.
- BAL, A. K., and R. R. GROSS. 1963. Mitosis and differentiation in roots treated with actinomycin. Science 139:584-586.
- BARTLETT, G. R. 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234:466-468.
- BASSARSKAYA, M. A. 1936. On the biochemical diagnosis of developmental stages in plants. Yarovizatsiya 6 (9):101-108.
- BONNER, J., and J. A. D. ZEEVAART. 1962. Ribonucleic acid synthesis in the bud an essential component of floral induction in Xanthium. Pl. Physiol. 37:43-49.
- BRUGOVITZKY, E., and I. BOSICA. 1967. Biochromatographic study of auxins and inhibitors in vernalized winter wheat. Flora (Jena) Abt. A. 158(2):206-223.
- ČAJLACHJAN, M. 1935. On the permeability of the plasma in the leaves of spring and winter wheats. C. R. Acad. Sci. URSS. 11:2.
- ČAJLACHJAN, M., and L. P. ZDANOVA. 1938. The role of growth hormones in formation. 11. Yarovisation and formation of growth hormones. C. R. Acad. Sci. URSS. 19:219-224.
- CASO, O. H., H. R. HIGHKIN, and D. KOLLER. 1960. Effect of gibberellic acid on flower differentiation in Petkus winter rye. Nature (Lond.) 185:478.
- CHAILAKHYAN, M. Kh. 1966. Role of gibberellins in photoperiodic processes and plant vernalization. Biol. Zh. Arm. 19(12):3-14.
- CHOLODNY, N. G. 1935. Über das Keimungshormon von Gramineen. Planta (Berl.) 23:289-312.

- CHOLODNY, N. G. 1936. On the theory of vernalization. C. R. Acad. Sci. URSS. N. S. 8:391-394.
- CHOLODNY, N. G. 1939. The internal factors of flowering. Herb. Rev. 7:223-247.
- CHOUARD. P. 1960. Vernalization and its relations to dormancy. Ann. Rev. Pl. Physiol. 11:191-238.
- COLLINS, W. T., F. B. SALISBURY, and C. W. ROSS. 1963. Growth regulators and flowering. III. Antimetabolites. Planta 60:131-144.
- CUTTER, E. G. 1965. Recent experimental studies of the shoot apex and shoot morphogenesis. The Bot. Rev. 31:7-113.
- DAVID, R. 1947. L'évolution des inclusion lipidiques du germe de blé pendant le traitement de printanisation. Compt. Rend. Acad. Sci. (Paris) 224:146-147.
- DAVID, R. 1951. L'influence de la printanisation sur les lipides de la plantule et de l'albumen de la semence de blé. Compt. Rend. Acad. Sci. (Paris) 233:428-430.
- DEL VALLE, M. R., and A. I. ARONSON. 1962. Evidence for the synthesis of stable information RNA required for bacterial spore formation. Biochem. Biophys. Res. Comm. 9:421-425.
- DENFFER, D. V. 1939. Über die Zusammenwirkung von Keimstimmung und täglicher Belichtungsdauer auf die Entwicklung von Sinapsis und Hordeum. Jb. wiss. Bot. 88:759-813.
- DEUEL, H. J. 1955. The lipids. Interscience Publishers Inc., New York 2:586.
- DEVAY, M. 1962. Biochemical processes in vernalization. Symposium on genetics and wheat breeding. Ag. Res. Inst. Hung. Acad. Sci. Martonvásár, Hungary. 17-40.
- DEVAY, M. 1964. The biochemical processes of vernalization. III. The changes in ascorbinoxidase activity in the course of vernalization. Proc. of a Sym. Praha - Nitra 6:149-152.
- DEVAY, M. 1965. The biochemistry of vernalization. IV. The changes of ribonuclease activity in the course of vernalization. Acta. Agr. Hung. Tomus XLV:275-287.

- DUNCAN, D. B. 1955. Multiple range and multiple F tests. *Biometrics* 11:1-42.
- DUPERON, R. 1949. Influence de la printanisation sur l'évolution des lipides et les glucides chez le *Sinapsis alba*. *Compt. Rend. Acad. Sci.(Paris)* 228:192-194.
- DUPERON, R. 1952. Effect of vernalization on metabolism. *Rev. Gen. Bot.* 59:580-631.
- EVANS, L. T. 1964. Inflorescence initiation in *Lolium temulentum* L. VI. Effects of some inhibitors of nucleic acid, protein, and steroid biosynthesis. *Aust. J. Biol. Sci.* 17:24-35.
- FEIERABEND, J., and R. BUENSOW. 1967. Promoting effect of chloramphenicol on the vernalization of winter rye. *Flora (Jena) Abt. A.* 158(1):153-156.
- FILIPPENKO, I. A. 1936. On the physiological characteristics of yarovised and unyarovised winter wheat. *C. R. Acad. Sci. URSS.* 3:185-189.
- FILIPPENKO, I. A. 1940. Inhibition of developmental processes in vernalized plants that have suffered partial anaerobiosis. *Compt. Rend. Acad. Sci. URSS.* 28:167.
- FINCH, L. R., and D. J. CARR. 1956. Nucleic acid content of Petkus rye embryos in relation to vernalization and devernialization. *Aust. J. Biol. Sci.* 9:355-363.
- FOLCH, J., M. LEES, and G. H. S. STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497-509.
- FRIEND, D. J. C., and F. G. GREGORY. 1953. Acceleration of flowering in partially vernalized grain of Petkus winter rye by subsequent treatment at high temperature. *Nature* 172:667-668.
- GARTON, G. A., M. MIHAILOVIĆ, M. ANTIĆ, and D. HADŽIJEV. 1963. Chemical investigation of wheat. *Glasnik Hemijskog Društva* 28:302-325.
- GASSNER, G. 1918. On physiological characteristics of spring and winter forms particularly of cereal plants. *Z. Bot.* 10:417-480.
- GAUGHRAN, E. R. L. 1947. The thermophilic microorganisms. *Bacterial Revs.* 11:189-225.

- GERLOFF, E. D., T. RICHARDSON, and M. N. STAHMANN. 1966. Changes in fatty acids of alfalfa roots during cold hardening. *Plant Physiol.* 41:1280-1284.
- GOTT, M. B., F. G. GREGORY, and O. N. PURVIS. 1955. Studies in vernalization of cereals. Xlll. Photoperiodic control of stages of flowering between initiation and ear formation in vernalized and unvernallized Petkus winter rye. *Ann. Bot. N. S.* 19:87-126.
- GREGORY, F. G., and O. N. PURVIS. 1936. Vernalization. *Nature* 138:249.
- GREGORY, F. G., and R. S. DEROPP. 1938. Vernalization of excised embryos. *Nature* 142:481-482.
- GREGORY, F. G., and O. N. PURVIS. 1938.a. Studies in vernalization of cereals. ll. The vernalization of excised mature embryos and of developing ears. *Ann. Bot. N. S.* 2:237-251.
- GREGORY, F. G., and O. N. PURVIS. 1938.b. Studies in vernalization of cereals. lll. The use of anaerobic conditions in the analysis of the vernalizing effect of low temperature during vernalization. *Ann. Bot. N. S.* 2:753-764.
- GROSS, P. R., and G. H. COUSINEAU. 1963. Effects of actinomycin D on macromolecule synthesis and early development in sea urchin egg. *Biochim. Biophys. Res. Comm.* 10:321-325.
- GRZESIUK, S., and K. KULKA. 1962. Mono- and oligosaccharides in vernalization process of winter rye grains. *Acta Soc. Botan. Polon.* 31:83-93.
- HÄNSEL, H. 1953. Vernalization of winter rye by negative temperatures and the influence of vernalization upon the lamina length of the first and second leaf in winter rye, spring barley and winter barley. *Ann. Bot. N. S.* 17:417-432.
- HARBERS, E., N. K. CHAUDHURI, and C. HEIDELBERGER. 1959. Studies on fluorinated pyrimidines. Vlll. Further biochemical and metabolic investigations. *J. Biol. Chem.* 234:1255-1262.
- HARRIS, R.V., and A. T. JAMES. 1965. Linoleic and α -linoleic acid biosynthesis in plant leaves and a green alga. *Biochim. Biophys. Acta* 106:456-464.

- HARTMAN, K. V., and C. HEIDELBERGER. 1961. Studies on fluorinated pyrimidines. XlII. Inhibition of thymidylate synthetase. J. Biol. Chem. 236:3006-3013.
- HATCHER, E. S. J. H. 1945. Studies in vernalization of cereals. IX. Auxin production during development and ripening of the anther and carpel of spring and winter rye. Ann. Bot. N. S. 9:235-266.
- HAYASHI, K., and Y. NAGATA. 1965. Effect of nucleic acid and its related compounds on flower initiation of Japanese radish. Gifu Daigaku Nogakubu Kenkyu Hokoku 21:105-109.
- HIGHKIN, H. R. 1955. Flower promoting activity of pea seed diffusates. Pl. Physiol. 30:390-391.
- HILDITCH, T. P. 1964. The chemical constitution of natural fats.. 4th ed. Chapman and Hall Ltd. London.
- HUBER, R. E., and SAUL ZALIK. 1963. Lipid and protein in germinating and developing flaxseed Linum Usitatissimum L. Can. Journ. Biochem. Physiol. 41:745-754.
- HUSSEY, G., and F. G. GREGORY. 1954. The effect of auxin on the flowering behaviour of Wintex barley and Petkus rye. Pl. Physiol. 29: 292-296.
- JACOB, F., and J. MONOD. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3:318-356.
- KAHAN, E., F. M. KAHAN, and J. HURWITZ. 1963. The role deoxyribonucleic acid in ribonucleic acid synthesis. VI. Specificity of action of actinomycin D. J. Biol. Chem. 238:2491-2497.
- KATES, M., and R. M. BAXTER. 1962. Lipid composition of masophilic and psychophilic yeasts (*Candida* species) as influenced by environmental temperature. Can. Journ. of Biochim. and Physiol. 40:1213-1227.
- KIHLMAN, B. A.. 1963. The effect of 5 - halogenated deoxyuridines on the frequency of x - ray induced chromosomal abberations in *Vicia Faba*. Hereditas 49:353-370.
- KIRK, J. M. 1960. The mode of action of actinomycin D. Biochim. Biophys. Acta 42:167-169.

- KLIPPART, J. H. 1857. An essay on the origin, growth, diseases, varieties, etc., of the wheat plant. Ann. Report Ohio State Bl. Agr. Sect. 12:562-816.
- KONAREV, V. G. 1954. The influence of vernalization on the behaviour of nucleoproteins and nucleic acids in grass embryos. Biokhimiya 19: 131-136.
- KONOVALOV, I. N. 1937. An experiment in yarovising the embryo of wheat seeds without endosperm. C. R. Acad. Sci. URSS. 16:381-383.
- KRUZHILIN, A. S. 1963. Accumulation of sugars in winter and summer wheat and in hybrids in relation to low temperatures. Fiziol. Rast. 10:374-376
- KRUZHILIN, A. S., and Z. M. SHVEDSKAYA. 1963. Physiological changes in seeds of winter wheat and of two-year plants during vernalization. Biol. Osnovy Povysheniya Kachestva Semyan Sel'skokhoz. Rast., Akad. Nauk. SSSR, Inst. Fiziol. Rast., Materialy Nauchn. Sessii, Moscow. 52-59.
- LANG, A., u G. MELCHERS. 1947. Vernalization und devernalization bei einen zweijahrigen planze. Z. Naturforsch 2b:444-449.
- LANG, A. 1957. The effect of gibberellin upon flower formation. Proc. nat. Acad. Sci. (Wash.) 43:709-717.
- LANG, A. 1965. Physiology of flower initiation. In: Encyclopedia of Plant Physiology. Springer-Verlag, Berlin XV:1380-1536.
- LASKOV, R., E. MARGOLIASH, U. Z. LITTAUER, and H. EISENBERG. 1959. High-molecular-weight ribonucleic acid from rat liver. Biochim. Biophys. Acta 33:247-248
- LASZTITY, R. 1966. Progress in the chemistry of wheat. 11. Lipids. Sutiopar 13 (2):64-68.
- LEATHES, J. B., and H. S. RAPER. 1925. The fats. 2nd. ed. Longmans, Green and Co., London 100,118.
- LEDOUX, L. 1963. Effect of X-rays on the translocation of labelled nucleic acids and proteins in germinating barley. Nuclear Science Abstracts 17 (10).
- LEDOUX, L., and P. GARLAND. 1963. Transport of nucleic acids and proteins through the cellular membranes. Protides Biol. Fluids, Proc. Colloq. 10:277-279

- LEDOUX, L. 1965. Uptake of DNA by living cells. Progress in nucleic acid research and molecular biology. 4:231-267.
- LEOPOLD, A. C., and K. V. THIMANN. 1949. The effect of auxin on flower initiation. Amer. J. Biol. 36:342-347.
- LEOPOLD, A. C., and F. S. GUERNSEY. 1953. Modification of floral initiation with auxins and temperatures. Amer. J. Biol. 40:603-607.
- LITTAUER, U. Z., and H. EISENBERG. 1959. Ribonucleic acid from *Escherichia coli* - preparations, characterization and physical properties. Biochim. Biophys. Acta 32:320-337.
- LOJKIN, M. 1936. Moisture and temperature requirements for vernalization of winter wheat. Contribs. Boyce Thompson Inst. 8:237-261.
- LONA, F., and A. BOCCHI. 1956. Caratteristiche d'accrescimento e sviluppo di alcune razze invernali di cereali trattate con acido gibberellico. Rev. int. Agricolt. 6:44-47.
- LYONS, J. M., T. A. WHEATON, and H. K. PRATT. 1964. Relationship between the physical nature of mitochondrial membranes and chilling sensitivity in plants. Pl. Physiol. 39:262-268.
- LYSENKO, T. D. 1928. The influence of the thermal factors on the duration of the developmental phases in plants. Trudy Azerbaidj. Op. St. 3:1-168.
- LYTTLETON, J. W., and G. B. PETERSEN. 1964. The isolation of deoxyribonucleic acid from plant tissues. Biochim. Biophys. Acta. 80:391-398.
- MARGULIES, M. M. 1962. Effect of chloramphenicol on light-dependent development of seedlings of *Phaseolus vulgaris*. var. Black Valentine, with particular reference to development of photosynthetic activity. Pl. Physiol. 37:473-480.
- MAXIMOV, N. A. 1934. The theoretical significance of vernalization. Bull. 16. Imp. Bur. Pl. Genet.: Herb. Pl.
- McMAHON, V., and P. K. STUMPF. 1964. Synthesis of linoleic acid by particulate system from safflower seeds. Biochim. Biophys. Acta 84:359-361.

- MECHAM, D. K., and A. MOHAMMAD. 1955. Extraction of lipids from wheat products. *Cereal Chemistry* 32:405-415.
- MELCHERS, G. 1936. Versuche zur Genetik und Entwicklungsphysiologie der Blühreife. *Biol. Zbl.* 56:567-570.
- MELCHERS, G. 1937. Die Wirkung von Genen tiefer Temperaturen und blühenden Pfropfpartnern auf die Blühreife von *Hyoscyamus niger* L. *Biol. Zbl.* 57:568-614.
- MELCHERS, G. 1939. Die Blühormone. *Ber. dtsch. bot. Ges.* 57:29-48.
- MELCHERS, G., u. A. LANG. 1941. Weitere Untersuchungen zur Frage der Blühormone. *Biol. Zbl.* 61:16-39.
- MELCHERS, G. 1952. The physiology of flower initiation. Lectures given at Imperial College of Science and Technology. University of London.
- MICHNIEWICZ, M. 1961. Influence of vernalization on the content of ascorbic acid in winter wheat. *Acta Agro botan. (Warsaw)* 10:119-131.
- MIHAILOVIĆ, M., G. A. GARTON, M. ANTIĆ, and D. HADŽIJEV. 1963. Chemical investigations of wheat. 5. Some chromatographic investigations of wheat lipids. *Glasnik Hemijskog Društva (Bulletin of the Chem. Soc. Belgrade)* 28(3-4):179-199.
- MOSKOV, I. and L. BOZOVA. 1962. Biochemical peculiarities of winter and spring barley. *Compt. Rend. Acad. Bulgare Sci.* 15:559-562.
- MURNEEK, A. E., and R. O. WHYTE; et al. 1948. Vernalization and photoperiodism: A symposium. *Chronica Botan. Co. Waltham, Mass.* 1-38.
- NAPP - ZINN, K. 1956. Zur Frage der Übertragbarkeit des durch die Vernalization bewirkten Blühimpulses. *Ber. dtsch. bot. Ges.* 69:193-198.
- NEUMANN, J. 1964. The effect of actinomycin D on lettuce seedlings and its differential uptake by roots and shoots. *Physiologia Plantarum.* 17:363-370.
- OOTA, Y. 1964. RNA in developing plant cells. *Ann. Rev. Pl. Physiol.* 15:17-36.

- PAVLOV, P., and L. TYANKOVA. 1962. Certain biochemical and physiological studies of vernalized and non - vernalized wheat seeds and plants. Izv. Nauchnoizslad Inst. po. Rastenievudstro, Akad. Selskostopansk. Nauki Bulgar. 14:67-30.
- POMERANTZ, Y., G. L. RUBENTHALER, R. D. DAFTARY and K. E. FINNEY. 1966. Effect of lipids on bread baked from flours varying widely in bread making potentialities. Food Technol. 20(9): 1225-1228.
- PURVIS, O. N. 1944. Studies in vernalization of cereals. VIII. The role of carbohydrates and nitrogen supply in the vernalization of excised embryos of Petkus winter rye. Ann. Bot. N. S. 8:285-314.
- PURVIS, O. N. 1948. Studies in vernalization of cereals. XI. The effect of date of sowing and of excising the embryo upon the responses of Petkus winter rye to different periods of vernalization treatment. Ann. Bot. N. S. 12:183-206.
- PURVIS, O. N., and F. G. GREGORY. 1952. Studies in vernalization of cereals. XII. The reversability by high temperature of the vernalized condition in Petkus winter rye. Ann. Bot. N. S. 16:1-21.
- PURVIS, O. N., and E. S. J. HATCHER. 1959. Some morphological responses of cereal seedlings to vernalization. J. Eept. Bot. 10:277-289.
- PURVIS, O. N. 1960. Effect of gibberellin on flower initiation and stem extension in Petkus winter rye. Nature. 185:479.
- PURVIS, O. N. 1961. The physiological analysis of vernalization. Encyclopedia of Plant Physiology. Springer-Verlag, Berlin XVI:76-122.
- RABSON, R., and G. D. NOVELLI. 1960. The incorporation of leucine C¹⁴ into protein by a cell free preparation from maize kernels. Proc. Nat. Acad. Sci. Wash. 46:484-488.
- RASUMOV, V. I. N. D. FEOKANOVA, and T. V. OLEINIKOVA. 1948. Vernalization von Wintergetreiden durch negative temperaturen. Dokl. Afad. Nauk. SSSR. N. S. 60:693-695.
- REJOWSKI, A. 1965. Effect of gibberellic acid on the vernalization of winter wheat seeds. Bull. Acad. Polon. Sci., Ser. Sci. Biol. 13(6):369-372.

- RICHTER, R. A. 1934. Practical solution of the problem of diagnosis of vernalization. Privoda, Lenin. 2:43-46.
- ROHRLICH, M., and Th. NIEDERAUER. 1967. Lipid - protein complexes in cereals. 1. Isolation of proteolipids from wheat, rye and oats. Fetter, Seifen, Austrichin. 69(4):226-230.
- ROUSER, G., G. KRITCHEVSKY, D. HELLER, and E. LIEBER. 1963. Lipid composition of beef brain, beef liver, and the sea anemone: Two approaches to quantitative fractionation of complex lipid mixtures. J. A. O. C. S. 40:425-454.
- SALISBURY, F. B., and J. BONNER. 1960. Inhibition of photoperiodic induction by 5 - fluorouracil. Pl. Physiol. 35:173-177.
- SARKAR, S. 1958. Versuche zur Physiologie der Vernalization. Biol. Zbl. 77:1-49.
- SECHET, J. 1962.a. Nucleic acids during vernalization treatment. Compt. Rend. (Paris) 254:3238-3240.
- SECHET, J. 1962.b. Biochemical modifications in seeds during vernalization. Compt. Rend. (Paris) 87:1069-1079.
- SHVEDSKAYA, Z. M., and A. S. KRUZHILIN. 1964. Oxidative metabolism and amino acid formation in cabbage buds during vernalization. Fiziol. Rast. 11(2):279-286.
- SLOTNICK, I. J. 1960. Mechanism of action of actinomycin D in microbiological systems. Ann. N. Y. Acad. Sci. 89:342-347.
- SPARMANN, G. 1961. Biochemische Untersuchungen uber. Vernalization und Blütenbildung der Gerste. 11. Planta 57:176-201.
- STOLETOV, V. N., E. V. BUDNITSKAYA, S. R. AGAMALOVA, T. A. KOKSHAROVA, and E. I. NIKITINA. 1965. Nucleic acid content of the seed embryos of spring, winter, and transition wheat forms. Izv. Akad. Nauk. SSSR. Ser. Biol. 30(6):836-847.
- STOUT, M. 1946. Relation to temperature of reproduction in sugar beets. Jour. Agr. Res. 72:49-68.
- STUMPF, P. K. and A. T. JAMES. 1963. The biosynthesis of long - chain fatty acids by lettuce chloroplast preparations. Biochim. Biophys. Acta. 70:20-32.

- SULLIVAN, B. 1940. The function of the lipids in milling and baking. *Cereal Chem.* 17:661-668.
- TAYLOR, J. H., W. F. HAVT, and J. TUNG. 1962. Effects of fluorodeoxyuridine on DNA replication, chromosome breakage, and reunion. *Proc. Nat. Acad. Sci. U. S.* 48:190-198.
- TELCHEROVA, L. 1964. Some metabolic changes in growth cones of wheat with drop in temperature. *Kletka i Temperatura Sredy* (Moscow - Leningrad:Nauka) Sb. 42-47.
- TERAOKA, H. 1967. Proteins of wheat embryos in the period of vernalization. *Pl. Cell Physiol.* 8:87-95.
- THIMANN, K. V., and R. H. LANE. 1938. After effects of the treatment of theseed with auxin. *Amer. J. Bot.* 25:535-543.
- TOMITA, T. 1958. Auxins in diffusate from vernalized winter rye. *Tohoka J. Ag. Res.* 9:63-67.
- TOMITA, T. 1959.a. A qualitative test by paper chromatography and paper electrophoresis on flower promoting substance in rye diffusate. *Proc. Crop Sci. Soc. Japan.* 28:150-152.
- TOMITA, T. 1959.b. The fractions of diffusate obtained from vernalized winter rye and their effect on flowering of annual meadow grass. *Tohoku J. Ag. Res.* 10:1-6.
- TOMITA, T. 1961. A vernalization like phenomom in winter wheat treated with flower - promoting substances. *Proc. Crop Sci. Soc. Japan.* 30:33-87.
- TOMITA, T. 1962.a. Studies on vernalization and flowering substance. 1. A detection of flowering substances contained in rye diffusate. *Tohoku J. Ag. Res.* 13(4):329-340.
- TOMITA, T. 1962.b. Studies on vernalization and flowering substance. 1. The effects of the extracts obtained from vernalized radish seedlings on the flowering of non - vernalized radish. *Proc. Crop Sci. Soc. Japan.* 31(2):163-166.
- TOMITA, T. 1963.a. Studies on vernalization and flowering substance. 11. Vernalization like phenomena in winter wheat and radish treated with flower promoting substances. *Tohoku Jour. Agric. Res.* 14(1):13-24.

- TOMITA, T. 1963.b. Electrophoretic isolation of flowering substances from vernalized plants and some vernalization - like phenomena caused by the application of extracts and of a pure substance. *Regulateurs Naturels de la Croissance Vegetable, Colloques Internationaux du Centre National de la Recherche Scientifique.* 123:635-648.
- TRIONE, E. J. 1966. Metabolic changes associated with vernalization of wheat. 1. Carbohydrate and nitrogen patterns. *Pl. Physiol.* 41:277-281.
- TSEN, C. C., I. LEVI, and I. HYLNKA. 1962. Rapid method for the extraction of lipids from wheat products. *Cereal Chem.* 39:195-203.
- VALUTA, G., and I. BRAD. 1960. Biochemical processes occurring at the vernalization of winter wheat in normal conditions and after its treatment with various substances. *Biochim. Zerna. Sb.* 5:87-99.
- WEISS, R. 1952. Changes in the principal forms of phosphorus during germination of seeds. *Bull. Soc. Chem. Biol.* 34:471-479.
- WELLENSIEK, S. J. 1962. The Control of Flowering. *Neth. J. Agric. Sci.* 10:390-398.
- WHYTE, R. O. 1949. Crop production and environment. Faber and Faber Ltd., 24 Russell Square, London. 11-230.
- WREN, J. J. 1960. Chromatography of lipids on silicic acid. *J. of Chromatography.* 4:173-195.
- ZEEVAART, J. A. D. 1962. DNA multiplication as a requirement for expression of floral stimulus in *Pharbitis nil*. *Pl. Physiol.* 37:296-304.
- ZENTNER, H. 1958. Flour lipids: A note on the acetone - soluble fraction. *Chem. and Ind.* 129-130.

B29888